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THE NEW YORK ACADEMY OF SCIENCES

SECTION OF PHYSICS AND CHEMISTRY

SOME DNA-DEPENDENT SYNTHETIC SYSTEMS OF ISOLATED CELL NUCLEI*

V. Allfrey and A. E. Mirsky

The Rockefeller Institute for Medical Research, New York, N. Y.

"The influence of the nucleus upon organic synthesis remains a fundamentally important fact. How this influence is exerted still remains almost unknown; but it is probably connected with exchanges of material between nucleus and cytosome which continually or intermittently take place."—E.B. Wilson.[1]

The conviction that the cell nucleus plays an active role in cell metabolism is based on a long history of biological experiment. Brandt in 1877 observed that enucleated fragments of *Actinosphaerium* soon died, while nucleated fragments could heal their wounds and continue to live.[1a] Subsequent studies of the ciliates *Oxytricha* (1884[2]) and *Stentor* (1885[3]) showed that the nucleus or a fragment of the nucleus was essential for wound healing, growth, and regeneration. Enucleation experiments have continued through the years and, repeatedly, the results have supported the conviction that the nucleus is a site of an intense activity essential to the continuing development and function of the cell. The effect of the nucleus is sometimes demonstrated in a very dramatic way, as in the experiments of Comandon and de Fonbrune,[4] which showed that motility is resumed immediately when a nucleus is reintroduced into cytoplasmic fragments of *Amoeba sphaeronucleus*.

The biochemical approach to nuclear activity often begins with methods for isolating cell nuclei. Many such methods exist. In earlier work in our laboratory, Alfred Mirsky and I were concerned with the comparison of the chemical compositions of cell nuclei isolated in different ways. Nuclei prepared in citric acid, sucrose, or saline solutions were compared with nuclei isolated in nonaqueous media by a modified Behrens procedure.[5-6] Nuclei prepared by the latter type of isolation were selected as "standards" because the nonaqueous methods prevent water-soluble materials from moving between nucleus and cytoplasm during the course of the isolation. It was shown that liver or kidney nuclei lost much of their protein when they were exposed to sucrose or citric acid solutions, simply by comparing them with the corresponding Behrens-type nuclei.[5-6]

Although many types of nuclei show a considerable loss of soluble proteins when they are exposed to isotonic sucrose solutions, it was found that calf thymus nuclei isolated in isotonic sucrose were the equivalent of the "standard" nonaqueous nuclei in many respects. The

*This paper, illustrated with slides, was presented at a meeting of the Section on October 7, 1958.

DNA analyses, protein composition, and enzymatic properties of the two preparations were in very close agreement. Once it had been established that thymus nuclei could withstand an isolation in a sucrose medium without leaching out their water-soluble components, it was decided to determine by test whether such nuclei had retained their capacity for complex synthetic reactions.

The isolation procedure employed is as follows. Fresh thymus tissue is minced with scissors and then homogenized at low speed in a blender in a 0.25 M sucrose-0.003 M $CaCl_2$ solution. The homogenate is filtered through gauze and flannelette to remove fiber and clumps of tissue. The nuclei are then separated by differential centrifugation and washed with sucrose-calcium chloride solution.

Under the light microscope such thymus nuclei, stained or unstained, appear remarkably clean. Contamination consists of a few whole cells, occasional red cells, and some cytoplasm that adheres to some of the nuclei. The extent of cytoplasmic contamination (as estimated by nucleic acid analyses and tests for cytochrome c oxidase activity) is found to be well below 10 per cent. Recent estimates of whole cell contamination in stained nuclear fractions place the number of intact cells at 3 per cent;[7] this is in accord with earlier whole cell counts made under the electron microscope.[8]

Tests for Nuclear Activity

The experiments about to be described are concerned with three types of synthetic reactions carried out by these isolated nuclear fractions: (1) the uptake of amino acids into nuclear protein; (2) the incorporation of purine and pyrimidine precursors into nuclear ribonucleic acids; and (3) the synthesis of ATP by the isolated nucleus. Some of the experiments will emphasize the role of desoxyribonucleic acid (DNA) in these nuclear activities; others will relate the synthetic capacities of the nucleus to its chemical environment.

Amino acid incorporation. When thymus nuclei are suspended in a buffered sucrose medium that contains radioactive amino acids and are incubated aerobically at 37° C., there is a rapid and considerable incorporation of isotope into the proteins of the nucleus. FIGURE 1 shows the time course of incorporation of lysine-2-C^{14}, alanine-1-C^{14}, and glycine-1-C^{14}. Curves similar to those shown for alanine or lysine are obtained with C^{14}-leucine, phenylalanine, tryptophan, and valine. These curves represent the incorporation of C^{14} into the total mixed proteins of the nucleus as a function of the time of incubation. They show a characteristic "lag" phase of 5 to 15 min., followed by a rapid incorporation of label into nuclear protein. After about 90 min. the rate of uptake falls off as some of the nuclei begin to autolyze and lose DNA.

These results represent an average incorporation for all the nuclei (plus the few cells) in suspension. To show that an isolated nucleus

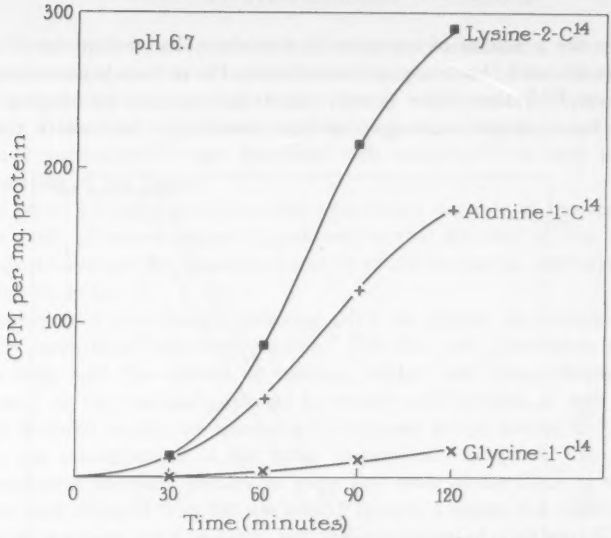

FIGURE 1. The time course of incorporation of alanine-1-C^{14}, glycine-1-C^{14}, and lysine-2-C^{14} into the proteins of isolated thymus nuclei. In this plot the data have been "normalized" to show the relative uptakes for equivalent amounts of the different amino acids, when each has the same specific activity in millicuries per millimole.

can indeed take up radioactive amino acids into its proteins, radioautographs were made, showing C^{14} labeling of single nuclei.[7,9]

The nature of the "lag" in the uptake of amino acids has been studied in several ways. In one series of experiments nuclei were preincubated for 10, 20, or 30 min. before adding the alanine-1-C^{14}. The time course of C^{14} incorporation by such preincubated nuclei was compared with that of "control" nuclei that received isotope at time zero. The results of the comparison are shown in FIGURE 2. It is apparent that a prewarming of the nucleus can diminish the lag phase without affecting the subsequent rate of C^{14}-alanine uptake. This suggests that an "activation" of some sort occurs during preincubation. There is now evidence that this activation is synchronous with ribonucleic acid (RNA) synthesis in the nucleus. Inhibition studies employing the substituted benzimidazole (5, 6 dichloro-β-D-ribofuranosyl benzimidazole, or DRB) have shown that this compound blocks RNA synthesis whenever it is added to isolated nuclei. This compound will also inhibit amino acid uptake provided it is added to nuclei at the outset of the lag phase. However, if the nuclei are first preincubated and DRB is added after the "lag," it has little effect on subsequent amino acid incorporation, although it still blocks RNA synthesis. This suggests that preincubation abolished the lag in amino acid uptake by permitting the synthesis of necessary ribonucleic acids.[9]

There are a number of experiments that show that the uptake of amino acids is related to nuclear protein synthesis. These have been summarized elsewhere;[9-10] they make it very clear that we are not dealing with adsorption or simple exchange reactions involving a few active centers

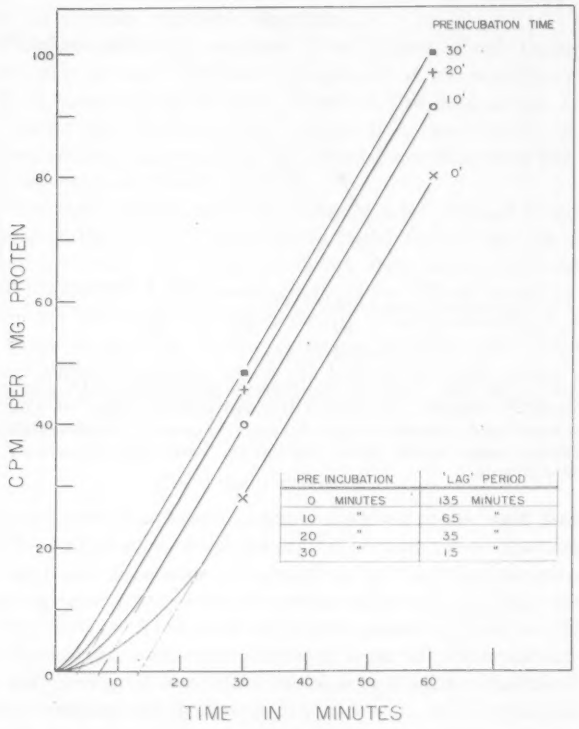

FIGURE 2. The effect of preincubation of thymus nuclei upon the subsequent incorporation of alanine-1-C^{14}. The nuclear suspension was preincubated for 10, 20, or 30 min. before adding the isotopic alanine. The time course of C^{14} incorporation by such preincubated nuclei is compared with that of control nuclei that received the isotope at time zero. Note that preincubation diminishes the lag period without affecting the subsequent rate of C^{14}-alanine incorporation.

The proteins of the nucleus can be fractionated in several ways following incorporation experiments. The specific activities of the different fractions have been compared.[9] This aspect of nuclear protein synthesis will not be presented here, except to observe that: (1) the histone proteins are poorly labeled; and (2) the protein fraction most closely associated with the desoxyribonucleic acid has a very high specific activity.

The role of deoxyribonucleic acid in protein synthesis. The theory originally suggested by Brachet[11] and Caspersson[12] that ribonucleic acids play a role in protein synthesis is now widely accepted as demonstrated. The effect of ribonuclease in suppressing amino acid uptake by liver microsomes[13] and bacterial cell residues[14] is very striking confirmation of the theory.

The ready availability of isolated cell nuclei capable of incorporating amino acids affords a unique opportunity to test the role of the deoxyribonucleic acids in the process of protein synthesis and in other synthetic systems as well.

A number of experiments relating DNA to protein synthesis in the nucleus have been described earlier.[9] For the most part these experiments deal with the effects of treating nuclei with deoxyribonuclease (DNAase) on the nuclear capacity for amino acid uptake. It was shown that if isolated nuclei are treated with DNAase before adding C^{14}-amino acids, the incorporation of the latter is seriously impaired. The degree of impairment becomes greater as more and more of the DNA is depolymerized and removed from the nucleus. FIGURE 3 shows the relationship observed between loss of DNA and the inhibition of alanine-1-C^{14} and lysine-2-C^{14} uptakes.

It should be pointed out that the sensitivity of the nucleus to treatment with deoxyribonuclease is further evidence for the absence of appreciable whole cell contamination, because intact cells are not sensitive (in minces and slices) to treatment with this enzyme.

DNA-substitution experiments. The ability of nuclei to incorporate amino acid is not irreversibly destroyed by treatment with deoxyribonuclease. It can be shown that the addition of a DNA supplement will restore much of their capacity for amino acid uptake.

The most striking observation made in "restoration" experiments is that DNA can be replaced by many other polyanions. It was first found that DNA preparations from widely diverse species could promote amino acid uptake just as well as the DNA of calf thymus (TABLE 1). The DNA molecule need not be intact, because alkali-denatured DNA and the larger split products obtained by enzymatic digestion of DNA can be fully as effective as the original DNA preparation. Even ribonucleic acids will substitute for the DNA of the thymus in restoring C^{14}-amino acid uptake into nuclear proteins. The lack of a specificity requirement was shown conclusively by experiments in which alanine-C^{14} and leucine-C^{14} uptakes were restored in DNAase-treated nuclei by the addition of polyadenylic acid (TABLE 2).

Although the spectrum of suitable DNA substitutes is a very broad one, it does not include a number of other related and perhaps equally likely compounds. For example, amino acid uptake is not restored by the free purine and pyrimidine bases or by mixtures of nucleosides. It was also observed that, although ribonucleic acid will substitute for

DNA, an alkaline digest of RNA that yields the component nucleotides has no effect; neither does an artificial mixture of the nucleoside 2′ and 3′ phosphates. Mixtures of the ribonucleoside-5′-phosphates, AMP, ADP, ATP, and a number of dinucleotides were also tested and found

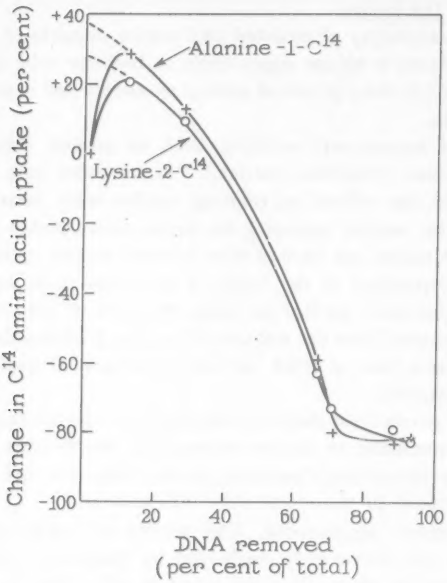

FIGURE 3. The effect of removing DNA from thymus nuclei upon the subsequent incorporation of alanine-1-C^{14} and lysine-2-C^{14}. Nuclei were pretreated with DNAase to remove increasing amounts of their DNA. They were subsequently incubated for 60 min. in the presence of the isotopic amino acid. The decrease in specific activity of the nuclear protein in treated nuclei, relative to that observed in untreated control nuclei, is plotted against the per cent removal of the DNA.

inactive. Among the dinucleotides tested without effect was adenylic-adenylic dinucleotide. This observation takes on added interest when it is compared with the previous finding that polyadenylic acid is an acceptable DNA substitute. Thus, the polynucleotide is effective where the dinucleotide is not, and molecular size emerges as one of the factors that determine whether a compound will substitute for DNA in restoring the activity of DNA-depleted nuclei.

Among other factors that determine the capacity of a polyelectrolyte to substitute for DNA is its electric charge. Polycations, such as polylysine or protamine, which carry a net positive charge at neutral *p*H values, cannot restore function. On the contrary, the addition of poly-

TABLE 1

EFFECT OF SUPPLEMENTARY DNAS IN RESTORING C^{14}-ALANINE UPTAKE IN DNAASE-TREATED NUCLEI

Supplement tested	*Specific activity of nuclear protein*			*Recovery of lost activity* %
	'Control' nuclei cpm/mg.	*DNAase-treated nuclei* cpm/mg.	*Treated nuclei + supplement* cpm/mg.	
1. Calf thymus DNA	91	25	70	68
Calf kidney DNA	91	25	70	68
Chicken erythrocyte DNA	91	25	60	53
Paracentrotus sperm DNA	91	25	72	71
2. Calf thymus DNA	135	21	93	63
Trout sperm DNA	135	21	89	60
3. Calf thymus DNA	145	69	101	42
Trout sperm DNA	145	69	101	42
Wheat germ DNA	145	69	86	22
4. Thymus DNA	99	33	101	103
Thymus DNA alkali-denatured	99	33	104	108
5. Thymus DNA	38	7.5	28	67
Thymus DNA, 'core' after DNAase digestion	38	7.5	24.5	56
Thymus DNA, dialyzable products of DNAase digestion	38	7.5	29	70

TABLE 2

EFFECT OF RIBONUCLEIC ACID AND POLYADENYLIC ACID SUPPLEMENTS ON AMINO ACID INCORPORATION BY DNAASE-TREATED NUCLEI

Supplement tested	*Amino acid*	*Specific activity of nuclear protein*			*Recovery of lost activity* %
		'Control' nuclei cpm/mg.	*DNAase-treated nuclei* cpm/mg.	*Treated nuclei + supplement* cpm/mg.	
1. Calf liver RNA	Alanine-1-C^{14}	92	54	80	68
Calf thymus DNA	Alanine-1-C^{14}	92	54	81	71
2. Yeast RNA	Alanine-1-C^{14}	68	23	58	78
Thymus DNA	Alanine-1-C^{14}	68	23	54	69
3. Polyadenylic acid	Alanine-1-C^{14}	450	167	263	34
Yeast RNA	Alanine-1-C^{14}	450	167	302	48
Thymus DNA	Alanine-1-C^{14}	450	167	273	38
4. Polyadenylic acid	Leucine-2-C^{14}	257	87	151	38
Yeast RNA	Leucine-2-C^{14}	257	87	152	38
Thymus DNA	Leucine-2-C^{14}	257	87	144	34

lysine to "control" nuclei that still retain their original DNA content leads to a drop in amino acid uptake.

These findings suggest a correlation between negative charge and the biochemical activity of the nucleus, but the question arises: Is electric charge alone a deciding factor or must a DNA substitute have a polynucleotide structure? This was tested in experiments in which large, negative molecules of a *nonnucleotide* nature were added to DNAase-treated nuclei[15]. It was found that polyethylene sulfonates (PS) were very effective in restoring alanine-1-C^{14} incorporation by DNAase-treated nuclei (TABLE 3). Other polyelectrolytes tested included heparin and chondroitin sulfate, both of which restored activity. Added serum albumin, serum globulin, or mixed plasma proteins had no effect on amino acid uptake by DNA-depleted nuclei. The ability of polyethylene sulfonate to substitute for DNA makes it evident that there is no specific requirement for a molecule of polynucleotide nature.

When restoration experiments are carried out in this way – first treating with the enzyme and, 30 min. later, adding a polyanion supplement – one usually observes that only a part of the lost nuclear activity is recovered. A DNA or RNA supplement will often restore about 70 per cent of the lost activity, but recoveries are sometimes as low as 30 per cent.

On the premise that the removal of DNA followed by a continued and prolonged incubation would lead to instability and irreversible changes in the state of the chromosomes, attempts were made to stabilize nuclear structure by substituting other polyanions for the DNA at the time of its removal[15]. This approach led to results summarized in FIGURE 4. It was found that, if sufficient polyethylene sulfonate is present at the time the DNA is removed, one obtains a nucleus that is the functional equivalent of "control" nuclei whose DNA is still intact. FIGURE 4 shows the relationship between the amount of polyethylene sulfonate present during DNAase digestion and the subsequent capacity of the nucleus for alanine-1-C^{14} incorporation. The total counts incorporated into protein are plotted against the amount of PS present during the initial DNAase-digestion. In all cases, regardless of the concentration of PS, 73 per cent of the DNA was made acid-soluble. It can be seen that nuclei given 3 to 4 mg. of polyethylene sulfonate reached a peak activity actually exceeding that of the "controls," despite the loss and removal of most of their DNA.

It should be stressed that in the intact nucleus it is the presence of DNA that makes possible the incorporation of amino acids into proteins. The substitution experiments that have been described merely indicate that the negative electric charge of the DNA molecule has a great deal to do with the synthetic activity of the nucleus.

Ribonucleic Acid Synthesis in Isolated Nuclei

It was shown previously that isolated thymus nuclei will incorporate orotic acid-6-C^{14} into RNA pyrimidines, and that both C^{14}-glycine and

adenosine-8-C^{14} will enter the ribonucleic acid purines.[9,16] As in the case of amino acid incorporation into nuclear protein, the uptake of labeled precursors into RNA is DNA-dependent. Treatment of the nucleus with deoxyribonuclease impairs its capacity for both orotic acid[9] and adenosine uptake.[15] Restoration is possible, and the list of suitable DNA substitutes ranges from other DNAs to polyethylene sulfonate.[15]

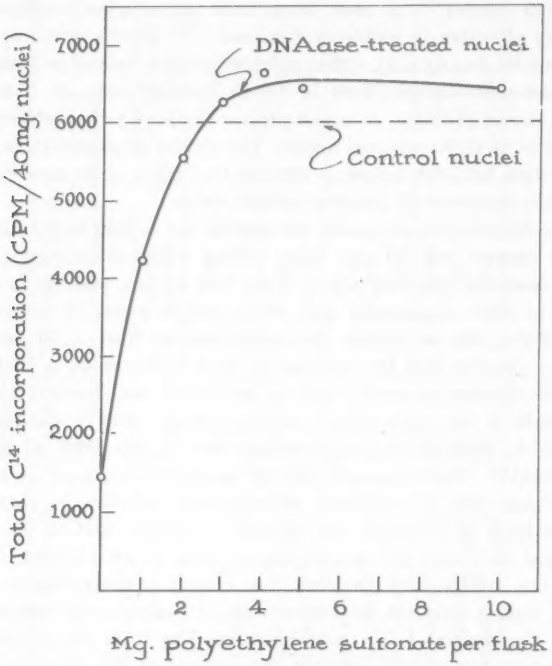

FIGURE 4. The effect of substituting nuclear DNA by increasing amounts of polyethylene sulfonate. All flasks contained 1.0 ml. of nuclear suspension (43 mg. of nuclei) and 0.4 ml. of 0.1 M glucose containing 3.75 mg. NaCl and 4.19 mg. $MgCl_2 \cdot 4H_2O$ per ml. plus the indicated amounts of polyethylene sulfonate. "Control" flasks received 0.5 ml. of 0.1 M sodium phosphate–0.25 M sucrose buffer at *p*H 6.7. Other flasks received this buffer containing 1.0 mg. of crystalline DNAase.

Studies of RNA synthesis in the isolated nucleus, as measured by the incorporation of both C^{14} orotic acid and adenosine-8-C^{14} have indicated a marked heterogeneity of the nuclear ribonucleic acids.[16] These experiments will not be described here, but it is of interest that the RNA of the nucleolus is far more active than the soluble and readily extractable RNA of the nucleus.

TABLE 3

EFFECT OF SUPPLEMENTARY POLYELECTROLYTES ON ALANINE-1-C^{14} UPTAKE INTO THE PROTEINS OF DNAASE-TREATED NUCLEI

*Supplement tested**	*Specific activity of nuclear protein*			*Activity lost*	*Activity regained*	*Recovery of lost activity*
	'Control' nuclei	*DNAase-treated nuclei*	*Treated nuclei + supplement*			
1. Thymus DNA	229	30	83	199	53	27
Heparin	229	30	122	199	92	46
Chondroitin sulfate	229	30	74	199	44	22
Polyethylene sulfonate (MW 27600)	229	30	138	199	108	54
Polyethylene sulfonate (MW 12900)	229	30	129	199	99	50
Polyethylene sulfonate (MW 5900)	229	30	102	199	72	36
2. No supplement	259	72	–	187	–	–
Polylysine	115	72	53	(43)	-19	0
Protamine	250	72	57	(178)	-15	0
Serum albumin	267	72	87	(195)	15	7.7

*Five mg. added in all cases.

Adenosine Triphosphate (ATP) Synthesis in Isolated Nuclei

From the experiments that have been described it is clear that DNA plays a central role in the biochemical activity of the intact nucleus. Its presence is essential for amino acid incorporation into protein and for adenosine and orotic acid uptake into RNA. Protein and RNA syntheses are, of course, dependent on a source of energy; amino acid activation requires ATP, and there is good evidence from other mammalian systems that nucleoside incorporation in RNA requires the intermediate formation of the nucleoside triphosphate.

Isolated thymus nuclei have been shown to be capable of phosphorylating the AMP that they contain to form ADP and ATP.[17] This process, although aerobic, differs in several important respects from the type of oxidative phosphorylation observed in mitochondrial suspensions. A comparison of nuclear and mitochondrial ATP synthesis will not be made here, but it should be stressed that thymus mitochondria prepared in the nuclear isolation medium (that is, sucrose containing calcium ions) do not synthesize ATP.

Agents that block ATP synthesis in the nucleus also block amino acid uptake into its proteins.[18] This observation fits in with new evidence that shows that thymus nuclear fractions contain amino acid-activating enzymes[19] that, like those described by Hoagland *et al.*,[20] need ATP for their action.

There are a number of experiments that relate the DNA of the nucleus to this capacity for ATP synthesis. The first of these is the demonstration that nuclei treated with desoxyribonuclease lose much of their ability to synthesize ATP.

This is shown in FIGURE 5, which depicts the separation of nuclear ATP from other nucleotides by ion-exchange chromatography on Dowex-1. A comparison of the "controls" (FIGURE 5A) with DNAase-treated nuclei shows that the latter have only about 30 per cent of the ATP found in intact nuclei. Restoration experiments with thymus DNA and yeast RNA both show a renewed capacity of the nucleus for aerobic phosphorylation (FIGURE 5C and 5D). It should be mentioned in passing that ATP synthesis in "restored" nuclei still requires the presence of oxygen; the addition of DNA in a nitrogen atmosphere has no effect.

It has recently been found that ATP synthesis can be restored to DNA-depleted nuclei by the addition of polyethylene sulfonate. Thus the nuclear capacity for ATP synthesis, like those for amino acid and adenosine incorporation, can be preserved by adding a polyanion of nonnucleotide nature.

Summary: Some General Conclusions and a Theory of Histone Function.

The experiments described on isolated nuclei show that at least three vital nuclear activities (ATP synthesis, amino acid incorporation into

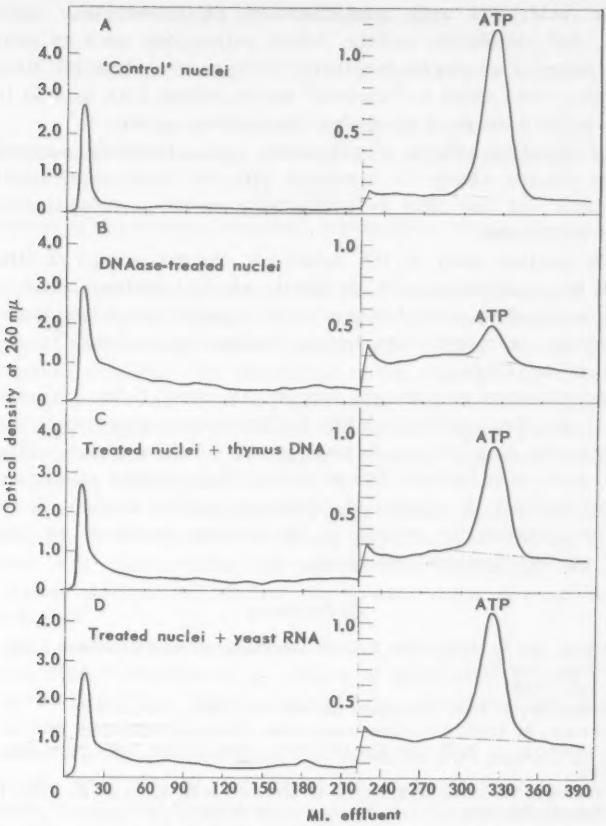

FIGURE 5. The chromatographic separation of ATP from other nucleotides in extracts of isolated cell nuclei. The optical density of the effluent E_{260} is plotted against the volume of solution passing through a column of Dowex-1 (formate). Curve A shows the pattern obtained from an acid extract of untreated thymus nuclei. Curve B shows the corresponding pattern obtained from nuclei pretreated with desoxyribonuclease. Note the decrease in the ATP peak. Curves C and D show the result of adding supplements of thymus DNA and yeast RNA to the DNAase-treated nuclei.

protein, and adenosine uptake into RNA) are DNA-dependent. Once the nuclei have been isolated, it is possible to remove as much as 75 per cent of their deoxyribonucleic acid content by treatment with deoxyribonuclease. Nuclei so depleted of their DNA lose most of their capacity for synthesizing ATP, protein, and RNA. The addition of polyanions restores function. The polyanions tested include DNAs, RNAs, poly-

adenylic acid, and such nonnucleotides as polyethylene sulfonate, heparin, and chondroitin sulfate. Added polycations such as protamine or polylysine do not restore function to DNAase-treated nuclei. Moreover, polylysine, when added to "control" nuclei (whose DNA is still intact) greatly inhibits amino acid uptake into nuclear protein.

The contrasting effects of polyanions and polycations suggest that negative electric charge is correlated with the biochemical activity of the nucleus and that DNA as a polyanion exerts a profound effect on nuclear metabolism.

In the nucleus many of the negatively charged groups of DNA are masked by combination with positively charged histones, and it may be that one function of the histones in the organization of the chromosome is to reduce or control chromosome function by masking the negative charges of the DNA.

Some preliminary experiments support this view. It has recently been found[21] that the addition of the highly basic arginine-rich histones (prepared from calf thymus nuclei) greatly inhibits amino-acid uptake into the proteins of isolated thymus nuclei. Thus, a basic protein prepared from the nucleus is capable of inhibiting nuclear activity. It remains to be seen whether the removal of the histones results in an increased rate of nuclear synthetic reactions.

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SECTION OF BIOLOGY

EXPERIMENTAL INFECTIOUS HEPATITIS STUDIES*

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The problem of viral hepatitis has been clearly formulated by Ward *et al.*[1] These authors state: "The control of viral hepatitis in both military and civilian populations presents one of the foremost problems in preventive medicine. The adaptation of the etiologic agent or agents to a laboratory animal or to tissue culture and the development of a specific immunologic test would provide tools to speed the solution of the problem. Neither tool is available at present. Man still remains the only established susceptible host."

The present paper is concerned with experimental aspects of infectious hepatitis (IH) with emphasis on efforts to induce the disease in mice. Only time will show whether the agents under discussion are related to the human disease and whether the present status of viral hepatitis will be altered.

The data are concerned primarily with the character of one agent (PG) that has produced hepatitis in two strains of mice since October 5, 1957. Other agents capable of inducing hepatitis have been obtained since the PG virus was discovered.

The effects of sera, whole blood, and feces from IH patients in laboratory animals were studied. Consistent with the experience of other investigators, no signs of disease were seen in the mice injected with these materials. The only time blind-passage technique was used was when liver and spleen emulsions from normal CFW mice were studied in an effort to determine the likelihood of activating a latent mouse hepatitis.

Since October 1957, liver biopsy material has been used exclusively as source material in isolation experiments.

The agent that produces hepatitis in mice has the following history. Whether each event recorded is an integral and contributing part in the isolation of a mouse virus remains to be seen. In other words, did the factor of human IH remain uncontaminated during the experimental procedures or did a contaminating virus enter the picture in place of, or together with, a mouse hepatitis?

*This paper, illustrated with slides, was presented at a meeting of the Section on October 13, 1958.

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On October 5, 1957, patient P.G. was seen by one of us (E.P.). He gave a history of approximately two weeks of nausea, vague gastric distress, tenderness on the right side, and constipation. The course of P.G.'s hepatitis can be seen in TABLE 1. Many clinically more severe cases than P.G. have been observed, but the degree of clinical involvement and discomfort of the patient are not necessarily indications for the best source of virus. Workers experienced in accumulating virus pools know that optimal virus potency may occur before pathological findings and onset of symptoms.

TABLE 1

HEPATITIS: PATIENT "P.G."

Symptoms and findings		*Weeks* 1 2 3 4 5 6 7 8 9 10
Anorexia		+ + + + + +
Vomiting		+ +
Jaundice		mild
Icteric index, units	100 - 80 - 60 - 40 - 20 - -	
Serum bilirubin, mg./100 cc.	8 - 6 - 4 - 2 - -	
Cephalin flocculation	4+ - 3+ - 2+ - 1+ - -	
Thymol turbidity, units	40 - 30 - 20 - 10 - -	
Pyruvic transaminase, units	1000 - 800 - 600 - 400 - 200 - 100 - -	

In the case of PG 2½ liver biopsies were put in glycerine and, about 3 hours later, without washing, were inoculated into a modified Maitland culture of whole chick tissue, the diluent consisting of 1 ml. of the culture medium. The remaining portion of the biopsy was studied microscopically and the pathological changes were considered characteristic of IH. This and other biopsies from the patients in the study will be reported on a clinical pathological basis. At the same time the biopsy was inoculated into a culture, between 1 and 2 ml. of clotted blood were inoculated into a sister culture.

The cultures containing PG biopsy material and blood were incubated for 6 days at 35° C. At the end of that time both cultures were pooled and, without further handling, were injected intraperitoneally in amounts of 0.5 ml. into ten 6- to 8-gm. P-mice, a strain resulting from a cross between C-57 and white Swiss mice. This strain, used since 1952, has been found to be at least as susceptible to virus infection as United States strains. The P-mice also have the advantage of being resistant to Salmonella infections.

For the first 5 days postinoculation the mice showed no symptoms. On the morning of the sixth day 1 mouse was found dead. Two of the animals had ruffled coats, walked on their toes, and appeared to be ill without presenting focal symptoms. The livers and spleens from the 1 dead and 2 sick mice were pooled and emulsified in a diluent of 10 per cent rabbit serum in water containing 5000 U. of penicillin and 5000 γ of streptomycin per ml., added in a ratio of 10 ml. per liver and spleen. After centrifugation in an International Centrifuge at 2000 rpm for 5 min. the cloudy supernatant was inoculated intraperitoneally into other P-mice in doses of 0.5 ml. per mouse. From that point it has been possible to transmit the hepatitis from mouse to mouse. The agent is now in its thirty-fourth serial mouse passage.

A fatal hepatitis associated with splenitis occurs in both P and CFW mice from 4 to 12 days after intraperitoneal injection of liver and spleen emulsions from sick animals. The incubation period varies directly with the concentration of infected material and is more consistent in mice weighing 8 to 10 gm. While the infection can be transmitted without difficulty to larger (15- to 20-gm.) CFW mice, the symptoms and subsequent deaths occur with greater irregularity than when small mice are used. Suckling mice have not been tested.

When freshly harvested liver and spleen emulsions from sick animals compose the source of virus, titers of approximately 10^{-7} are obtained. When the virus is stored as a 20 per cent tissue emulsion at -30° C. there is a loss of 1 or 2 logs of potency in 2 to 4 weeks. Since the PG agent is relatively new, efforts have been directed toward maintaining potent pools rather than following a quantitative deterioration curve.

Mice dying or freshly dead have been frozen *in toto* and kept at -30° C. Livers and spleens from dying animals have been stored in 50 per cent

neutral glycerine or at -30° C. as 20 per cent emulsion pools in the following diluents: 5 or 10 per cent normal rabbit serum; Eagle's vitamin amino-acid-glutamine in Hanks' BSS; Medium No. 199; water; 10 per cent calf serum in water; 10 per cent horse serum in water; and skimmed milk. Although not all samples have been tested after equal lengths of storage, it is clear that the mouse frozen *in toto* retains considerable virus potency after at least 8 months at -30° C., that is, $10^{-4.5}$, the limit of the test. The glycerine samples have retained activity over the longest period tested, that is, 10 months. The pools, regardless of the diluent, have retained considerable activity for at least 4 months.

In addition to the intraperitoneal routes, mice have been successfully injected subcutaneously and intracerebrally with the PG virus. This laboratory host also is susceptible to infection by feeding. In the intracerebrally infected animals death appeared to be due at least in part to central nervous system involvement, since ataxia and an occasional convulsion were noted. However, the livers, spleens, and brains of these animals produced infection when injected by both intracerebral and intraperitoneal routes.

In 3 experiments rabbits were injected intracerebrally and by corneal scratch with approximately 100,000 mouse doses of the PG agent. In one experiment rabbits were injected with a similar amount of virus subcutaneously and intraperitoneally. No sign of infection was noted in any of the rabbits that received the large amounts of virus, regardless of the route of inoculation. All rabbits were inbred New Zealand White strain, weighing 3 to 3½ lb. and were approximately 8 to 9 weeks old. Guinea pigs were similarly resistant to injection in the foot pad.

In view of the importance that has been placed on the resistance of the human IH virus to heat, this factor was studied with the PG virus. In multiple experiments with PG virus heated to 52° C., 54° C., and 60° C. the following results were obtained: there was no loss of virus after exposure to 52° C. for 30 min. At 54° C. there was a reduction in virus activity from $10^{-7.63}$ to $10^{-6.31}$. At 56° C. the virus survived for 20 min., and variable results were obtained after heating for 30 min. We have therefore concluded that there is little loss of virus activity after heating for 30 min. at 54° C. and that the PG agent survives 20 min. at 56° C., but its reactivity at the same temperature for 30 min. is questionable. The PG virus is destroyed within a few minutes after exposure to 60° C.

It is our impression, based on conversation with research workers in the field of hepatitis, that the heat stability of the human IH virus is considerably greater than the 30-min. exposure to 56° C. that has been reported.[2] We have not found a reference to heat stability longer than 30 min.

A comprehensive study of the effect of *p*H on PG virus has not been done. However, tests carried out at *p*Hs approximating 5, 7, and 8 show that the potency of the virus is not affected at the *p*Hs studied.

The PG virus is not propagated on the chorioallantoic membrane. In multiple tests carried out for 3 egg passages no plaques were seen and emulsions of chorioallantoic membrane of each of 3 egg passages did not produce disease in mice. In the fertilized egg experiments the virus had no effect on the development of the embryo or on the supporting membranes.

At various intervals infectious material from liver-spleen pools have been tested on routine bacteriological media, as well as in 10 per cent horse serum medium. There have been no indications of the presence of bacteria or of pleuropneumonia organisms.

A study of PG activity in tissue culture is in a preliminary stage. Virus from early mouse passages was propagated in Maitland chick preparation and a gross six-day cytopathic effect was noted, together with infectivity values of more than $10^{-4.5}$ mouse LD_{50}s. The CPE was not seen after the fourth serial culture generation. Virus could be demonstrated in mice through the eighth serial culture generation, but infectivity was lost when another test was run with the eleventh culture generation. Calculations of initial inoculum dilution factors (10^{-10}) made it clear that propagation had occurred. It is not clear why the virus was not maintained in serial chick cultures indefinitely and it is of interest that we had a similar experience with chick cultures in Havana.

Repeated attempts to propagate PG on rabbit kidney have failed. There was no CPE in rabbit kidney cells even in the first generation with an inoculum of 0.1 ml. of 10^{-2} material from a PG pool, with a mouse LD_{50} of $10^{-6.5}$. After freezing and thawing sister cultures have been injected into mice without producing disease.

At this time it is not clear whether the PG agent will grow in monkey kidney cells. Infectivity of the tissues from mice was noted in the first culture generation, but this action was probably due to the initial inoculum. No clear-cut CPE changes that could be specifically and unquestionably attributed to the virus under study were noted in the monkey kidney cells. Serial passages are under study, and preliminary data suggest that the virus does not grow in the monkey kidney cells. The action of the virus on human liver cells (Chang), both in relation to CPE and mouse infectivity, is promising. Other cell lines that are being studied in relation to the PG virus are HeLa, Marioni spleen, Marioni heart, mouse embryo, and bovine kidney.

In an effort to clarify the status of the PG virus considerable effort was expended in developing a serologic test, since it was not known whether the agent under study would react with human serum. The neutralization technique was selected because of its broad application in virus diseases and because the PG virus produced death in mice, so that comparative LD_{50}s with known controls and unknown sera could be evaluated.

A standardized neutralization technique has been developed using 0.25 ml. amounts of serial dilutions of virus mixed with an equal amount

of undiluted serum and then incubating the mixtures for 2 hours at 37° C., followed by an additional 20 hours of incubation at -4° C. At the end of this period the serum-virus mixtures are diluted tenfold and injected into mice intraperitoneally in amounts of 0.25 ml. In an effort to conserve the amount of serum and also avoid the dilution factor, serum-virus mixtures of 0.1 ml. per mouse inoculum are under study.

As in the case of other virus diseases, the serologic results have been considered significant if two logs of virus are neutralized by test sera.

It was obviously desirable to study paired sera collected from IH patients in the acute and convalescent phase of the disease in order to determine whether there was a rise in antibody titer in neutralization tests. Considerable difficulty was experienced in obtaining such sera, and it is hoped that acute and convalescent sera in code will be available. Neutralization results with the paired sera that we were able to obtain are seen in TABLE 2. It is to be emphasized that, while strict code technique was not followed, the diagnosis in each instance was unknown to us and the actual tests were run under "blind" conditions. In TABLE 2 a rise in neutralization index is seen in most instances. Only additional tests will reveal the degree of significance in this field.

TABLE 2

"PG" NEUTRALIZATION TESTS IN MICE
PAIRED SERA: INFECTIOUS HEPATITIS AND OTHERS

Patient	*Serum collected*	*Log difference between test serum & control*	*Neutralization indices*	*Diagnosis*
I.A.	2/12/58 3/13/58	3.02 4.02	1,050 10,500	IH
T.B.	3/25/58 4/8/58	1.30 2.42	20 263	IH
P.G.	10/5/57 2/26/58	1.23 2.67	17 468	IH
M.F.	3/26/58 4/18/58	0.0 1.3	0 20	IH Volunteer
M.G.	2/21/58 6/5/58	2.21 2.55	163 355	IH
I.H.	2/26/58 9/11/58	0.3 1.0	1 10	IH
A.G.	4/22/58 5/7/58	0.35 3.3	22 2,000	IH
J.C.	7/31/58 8/7/58	1.35 0.65	None	Mumps
H.R.	7/31/58 8/7/58	0.95 0.35	None	Mumps

Difficulties were also encountered in accumulating sera drawn at proper intervals. Again it should be noted that the diagnoses were unknown.

In TABLES 2, 3, and 4 results have been summarized where PG virus has been the antigen and neutralization tests have been run against sera from patients who have suffered from IH or other diseases. Neutralization results in the convalescent phase have been borrowed from the paired sera in an attempt to show all data available at this time. Sera from 19 convalescent IH humans have been tested under standard conditions (TABLE 3). In 7 instances the neutralization index of 100,

TABLE 3

"PG" NEUTRALIZATION TESTS IN MICE:
POSTACUTE INFECTIOUS HEPATITIS SERA

Patient	*Serum collected after acute onset*	*Test serum vs. control (log difference*)*	*Neutralization Index*
I.A.	15 weeks	4.02	10,500
T.B.†	7 weeks	2.42	263
P.G.	21 weeks	2.67	468
M.F.	5 weeks	1.3	20
F.G.‡	12 weeks	0.0	0
M.G.	14 weeks	2.55	355
I.H.	32 weeks	1.0	10
A.G.	3 weeks	3.3	2,000
M.G.S.†	13 weeks	1.46	29
H.P.†	1 year	0.93	8.5
V.S.	2 weeks	2.5	317
H.S.†	5 years	4.0	10,000
J.E.S.	4 weeks	1.0	10
E.R.	6 weeks	2.48	302
I.I.P.†	1 week	2.65	447
M.K.†	10 weeks	2.55	355
J.E.	2 years	1.3	20
P.O.R.§	25 weeks	Under test	
A.C.G.	12 weeks	Under test	

* Based on LD_{50}.
† Miami, Fla. cases. All others from Havana, Cuba.
‡ Father of M.G.
§ Third virus isolation.

which has been considered significant, has not been obtained. Two of these sera are of interest. M.F. received the PG virus intraperitoneally and the specimen of April 18 represents the first of several sera that have been collected. Furthermore, since the initial use of PG virus induced neither symptoms nor signs of liver dysfunction, another dose has been administered by mouth. It is reasonable to expect that the titer for the PG virus in serum MF should rise with a second administration and longer intervals between neutralization tests. F.G. is the father of

TABLE 4

"PG" NEUTRALIZATION TESTS IN MICE:
SERA FROM CASES OTHER THAN INFECTIOUS HEPATITIS

Patient	*Diagnosis*	*Test serum vs. control (log difference)*
G.C.	Cirrhosis	-0.2
M.H.*	Cirrhosis	0.18
F.P.	Blood donor†	0.0
M.S.V.	Fever unknown origin	0.6
M.L.S.	IH family contact	0.93
A.D.G.	Liver necrosis (toxic)	0.7
J.R.	Typhoid fever	-0.42
J.C.C.	Cirrhosis	0.15
D.D.A.	Typhoid fever	-.03
E.Z.	Cirrhosis	0.5

*Second virus isolation.
†Suspected of transmitting serum hepatitis.

patient M.G. Insofar as can be determined, both cases were IH and the assumption was made that the father was the source of infection for the daughter. We have no explanation for the lack of antibodies in the father compared to the neutralization index of 355 for the daughter. In the remaining sera tested neutralization indices against PG range from 263 to 10,500. Six of these were from paired sera, and 5 of the 6 showed what appears to be a significant rise in antibodies. In TABLE 4 serum from 10 patients with diagnoses other than IH did not give neutralization indices of significance. Attempts are being made to trace the cirrhosis patients to determine if a factor of IH entered the clinical picture. Patient M.H. in this group is of interest since he has periodically returned to the hospital in Havana with recurrent attacks of hepatitislike symptoms. An agent that may have been obtained from his liver biopsy is antigenically unrelated to PG. The hepatitis in mice produced by the MH virus is different from the PG infection in incubation period, mortality, and pathology.

A study of 10 volunteers who received 400,000,000 mouse LD_{50}s of PG orally was initiated September 1, 1958. There have been no ill effects, and periodic serum samples, including control sera, have been collected. This will be the subject of another paper. At the present time the purpose of the study can be stated. With multiple administrations of PG virus correlated with rise in antibodies, a mechanism will be available to check the specific relationship of PG virus with human IH by an eventual challenge using the latter agent. If the antibody rise is rapid and reaches a high level, consideration can be given to a vaccine against the human disease, provided the volunteers withstand infection.

The activity of human gamma globulin prophylactically in IH has been well established by Stokes *et al.*,[3] as well as by Ward *et al.*[1] For this reason gamma globulin was introduced in the neutralization tests against PG. In 4 experiments human GG has neutralized the PG virus with a neutralization index range of 500 to 3200. In terms of logs of virus neutralized this would be equivalent to $10^{-2.7}$ to $10^{-3.5}$. Gamma globulin is now routinely included in all neutralization tests.

It is a matter of general knowledge that the herpes virus is also neutralized by gamma globulin pools. On the basis of personal communications from both Vladimir Kubelka in Czechoslovakia[4] and F. O. MacCallum of England,[5] we believe a virus that produces hepatitis in mice and has been isolated in Czechoslovakia may be related to herpes simplex. In view of these statements, the PG virus was tested against antiherpes serum and the results, shown in TABLE 5, indicate that whatever the nature of PG may prove to be, it does not appear to be a member of the herpes group.

TABLE 5

"PG" NEUTRALIZATION TESTS IN MICE:
COMPARISON BETWEEN GAMMA GLOBULIN AND ANTIHERPES

Test material	*Virus titer LD_{50}*	*Neutralization indices*
Normal rabbit serum	7.0 7.0	–
Antiherpes rabbit serum	6.7	2
Gamma globulin	3.5	3170

Additional data to prove the lack of relationship between PG virus and herpes are as follows: failure of PG virus to infect rabbits by all routes; and the incapacity of the agent to produce infection and lesions on the chorioallantoic membrane. Furthermore, the classic acidophilic intranuclear inclusion bodies of herpes are absent in the livers and spleens of affected mice studied microscopically.* Finally, even though the PG tissue culture studies are in a preliminary state, sufficient information is available to differentiate them from the familiar pattern of herpes virus *in vitro*.

*Ninety-six mice injected with PG virus have been studied by Max Millard, Department of Pathology, University of Miami Medical School. While this phase of the project will be reported as a separate entity, it is pertinent that intracytoplasmic acidophilic inclusion bodies were noted and there were no intranuclear inclusions suggestive of herpes.

With the degree of neutralization exerted by human gamma globulin, it was not surprising that difficulty was experienced in using human serum from so-called normal individuals as controls in neutralization tests. Normal rabbit serum, calf serum, and horse serum have been satisfactory control sera.

In addition to PG, 2 stable strains of virus that produce hepatitis in mice are under study. The second isolation has been labeled M.H., whose serum-PG test appeared in TABLE 4. The diagnosis on this patient is cirrhosis and his serum failed to neutralize PG. The technique for handling the MH material isolation was similar to that followed in the case of PG, except that no whole blood was used and only one liver biopsy was inoculated after emulsion into the modified Maitland culture. Following incubation for 8 days at 35° C., the culture was emulsified and injected into 6 mice. At the same time one half cc. of the lightly centrifuged (2000 rpm) material was inoculated into other cultures. Two lines of this virus are available. The first came from a mouse that was ill 2 weeks after receiving the original culture biopsy material. The second line of virus came from the fourth culture generation passed serially at 8-day intervals. It is interesting that only 1 of the 6 P-mice that were inoculated with the first culture showed signs of illness. In contrast, all of the 6 mice that received the fourth culture generation were found dead or were very ill on the twelfth day postinoculation and were sacrificed for passage. The incubation period in the MH series has varied from 12 to 30 days following intraperitoneal injection. In its nineteenth passage the incubation period now has been reduced to 8 to 10 days when 1:10 liver-spleen emulsions are used as inoculum.

Another facet of the MH inoculation may be important in reference to the tissue cultures. In the first four culture generations a marked, grossly visible CPE was observed on the eighth day after inoculation with the preceding culture. In a manner similar to the experience of PG the CPE was lost and the MH virus was not maintained in tissue culture. There is no difficulty in transmitting the MH virus from mouse to mouse.

The MH strain is not as lethal as PG, and even severely ill animals with ascites occasionally recover. Such animals have been reinoculated with the homologous agent after 30 days, have passed through the same cycle of illness and ascites, and the procedure has been repeated for a third time, with the same results. The pathology in the livers and spleens of such animals is predominantly fibrotic. The pathological diagnosis has been cirrhosis.

The third isolate has been labeled POR; again, a single liver biopsy was used in tissue culture. After inoculation of the culture material intraperitoneally into mice, 2 of 6 animals were found dead 3 weeks later. The livers and spleens have produced infection in serial mouse passage in a fashion resembling PG. The incubation period for POR,

now in its twelfth mouse passage, has been reduced to 4 to 6 days when a 1:10 liver-spleen emulsion is inoculated intraperitoneally.

It is now common knowledge that latent mouse viruses have been demonstrated in England by Gledhill and Andrewes[6] and in the United States by Jordon and Mirick,[7] Nelson,[8] and Braunsteiner and Friend.[9]

It is our impression that the agents discussed here are not the same as the English and Nelson's strains, which have high lethality for mice only when the virus is present in the animal along with *Eperythrozoon coccoides*. Many of our suspensions have been stored or injected in the presence of tetracycline antibiotics and these substances have also been administered to the mice before and after infection. The PG virus has not been affected by the tetracyclines. Furthermore, Giemsa-stained blood smears from both strains of mice under study have not shown the presence of any organism whatsoever. The strain at Sloan-Kettering Institute, New York, N. Y., which is being studied by Charlotte Friend appears to be different from our agents on the basis of heat reactivity. In one study with that virus, activity was present after 30 min. exposure to 80° C.[10] This finding has been confirmed.

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SECTION OF MATHEMATICS AND ENGINEERING

THE METALLURGY OF A LIQUID FUEL REACTOR*

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Fluid fuels are being studied for reactor applications because there is a promise that their development will lead to a considerable reduction in the cost of producing electric power from fission heat. Solid fuel elements are limited in burn-up by radiation damage and build-up of fission product poisons. The cost of remote refabrication is expected to be high. Since no radiation damage to fluid fuels is anticipated, there should be unlimited burn-up in such systems. If chemical processing is done on a continuous or batch basis, the loss of neutrons by capture in the fission products is determined only by the processing costs. One serious problem in the fluid fuel reactor designs that is absent in solid fuel reactors is the circulation of radioactive fuel to an external circuit, including heat exchangers, pumps, and other such units. This introduces additional problems of costly remote maintenance and corrosion prevention in the total system.

A liquid metal fuel reactor (LMFR) employing a uranium-bismuth fuel (UBi) is being studied by the Nuclear Engineering Department of the Brookhaven National Laboratory. In the reactor proposed, a liquid uranium bismuth fuel would be circulated through a graphite core and then through a heat exchanger (FIGURE 1). The concentration of uranium in the bismuth and the dimensions of the core moderator are such that a fission chain reaction occurs in the core. Fission heat generated in the core is transported by the fuel to the heat exchanger, where it is transferred to a secondary working fluid which in turn produces steam to drive a turbine. The core inlet and outlet temperatures of a proposed cycle are 425 and 500° C., respectively.

Around the core of an LMFR breeder there would be a blanket in which a fertile material such as a dispersion of a thorium compound in bismuth can by absorption of neutrons be converted into a fissile material:

$$Th^{232} + n \rightarrow Th^{233} - \beta^- \rightarrow Pa^{233} - \beta^- \rightarrow U^{233}$$

The fuel processing scheme under development by the Chemical Engineering Division would remove most of the high cross-section fission products (rare earths) by contacting the UBi alloy with an oxidizing agent such as bismuth trichloride dissolved in the ternary eutectic of $MgCl_2$, KCl, and NaCl. The fission product xenon would be purged

*This paper, illustrated with slides, was presented at a meeting of the Section on October 17, 1958.

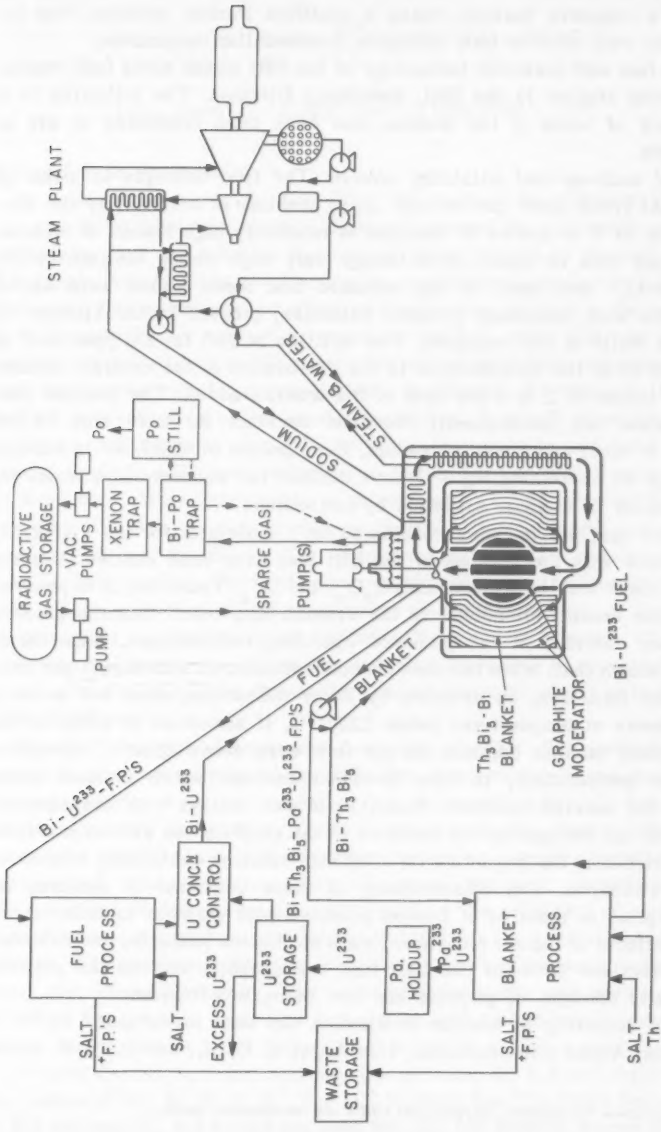

FIGURE 1. Liquid metal fuel reactor. Schematic diagram showing reactor, steam plant, and chemical processing.

from the solution with helium or by a vacuum degassing treatment. The removal of the less chemically active fission products – Ru, Rh, Pd, and Te – appears feasible using a modified Parkes process, that is, reaction with zinc to form insoluble intermetallic compounds.

The fuel and materials technology of the UBi liquid metal fuel reactor are being studied in the BNL Metallurgy Division. The following is a summary of some of the studies that have been completed or are in progress.

Fuel make-up and solubility effects. The first attempts to make up the fuel [1000 parts per million (ppm) uranium in bismuth] by the dissolution of U in molten Bi resulted in relatively high losses of uranium – 50 per cent or more – even though very high purity bismuth (99.99 per cent),* was used. It was assumed that these losses were due to reaction with impurities (oxides, chlorides) present in the bismuth or on the walls of the crucibles. The addition of 200 to 300 ppm each of Mg and Zr to the bismuth prior to the dissolution of the uranium reduced these losses to 2 to 5 per cent of the uranium added. The uranium concentration has subsequently remained constant for more than 16,000 hours in steel and graphite vessels. Experiments in which air is bubbled through Bi containing Mg + U have defined the concentration limits for prevention of uranium oxidation by magnesium.

Since graphite is being considered as a moderator for the LMFR, its reactions with the uranium in the UBi fuel have been studied. Uranium forms three stable carbides, UC, U_2C_3, and UC_2. Therefore, it is possible that the uranium dissolved in the bismuth may react with the graphite to form carbides at the proposed operating temperatures. Experiments have shown that, when the uranium concentration is less than 1 per cent, carbide formation, as detected by X-ray diffraction, does not occur in 100 hours at temperatures below 1200° C. If zirconium is added to the UBi fuel, uranium carbides do not form even above 1200° C.: zirconium reacts preferentially to form zirconium carbide, which is more stable than the uranium carbides. Reaction of the uranium with the adsorbed oxygen and nitrogen on the surfaces of the graphite can also be prevented by pretreating the graphite with a bismuth solution containing magnesium and zirconium. The effectiveness of these additions in reducing the adsorption or reaction of fission products with graphite is under study. The effects of reactor radiations, such as fission products, and neutrons, on reactions between the UBi fuel and graphite and on the physical property changes of graphite are now being investigated.

The solubility of uranium in bismuth has been investigated by D. W. Bareis, Atomics International, Los Angeles, Calif., and by J. R. Weeks

*Analyses for chlorine, oxygen, and sulfur are not normally made.

and D. G. Schweitzer, both of Brookhaven National Laboratories. The solubility up to 725° C. is given by:

$$\log (\text{ppm U}) = 6.586 - \frac{224°}{T} (\pm 2\%) \quad (300 - 480° \text{C.})$$

$$\log_{10} (\text{ppm U}) = 7.263 - \frac{2744}{T} (\pm 10\%) \quad (480 - 725)$$

where T is in degrees Kelvin.

In a proposed reactor, the fuel (UBi) will contain the additives Mg and Zr, as well as fission products and constituents of the containing vessels and piping that are soluble. Magnesium, when present in the fuel up to 1.0 weight per cent, has no effect on the solubility of uranium in bismuth. Zirconium has a very marked effect on the uranium solubility, depressing it to roughly 75 per cent of its value at 425° C. when 200 ppm of zirconium are added to the UBi alloy. Nickel, an important constituent of high temperature steels, is very soluble in bismuth. As little as 10 ppm results in a reduction of the uranium solubility. Iron and chromium, also important constituents of steel, are much less soluble and have little effect. A slight additional reduction in the uranium solubility was noted in some exploratory experiments to determine the effect of mixed fission products (130 ppm consisting of approximately 75 ppm Nd, 15 ppm Sm, 8 ppm Ru, 20 ppm Sr, 15 ppm Cs) in conjunction with the inhibitors (250 ppm Zr, 350 ppm Mg). These studies on solubility effects determine what type of alloys can be selected as materials for containment of the fuel system, what concentrations of additives (Mg, Zr) can be used, what the minimum fuel temperature must be, and what frequency of chemical processing is necessary to maintain the desired composition of the fuel in a fully liquid state.

Materials for containment and corrosion studies. The selection of materials for containment of the UBi fuel system was based primarily on two criteria: Were its properties (physical and chemical) adequate for the proposed application (550° C. max.), and was there sufficient successful experience with it to warrant its use as a reactor construction material? It was necessary also to regard economic considerations and the effect of dissolution of constituents on the solubility of uranium. Molybdenum and tantalum were eliminated except for specialized applications because they did not meet the above requirements, although tests had shown that they were extremely insoluble and resistant to corrosion by the UBi fuel. The low-chrome and carbon steels were selected for evaluation. The former contain 0.1 to 0.15 per cent C, 1 to 5 per cent Cr, 0.5 to 1 per cent Mo. 0.5 per cent Si, 0.4 to 0.6 per cent Mn, traces of Ni, Al, P, S, N_2, and O_2, balance Fe. The latter have 0.1 to 0.3 per cent C, 0.4 to 0.8 per cent Mn, 0.4 per cent Si, traces of Al, P, S, N_2, O_2, balance Fe.

Two methods of testing were employed: static (isothermal) and dynamic (nonisothermal). The static corrosion tests, in which samples of the above steels were immersed at 550° C. in UBi and in UBi containing Mg and Zr, showed that all the steels were attacked by the former but not by the latter solution if sufficient (50 to 100 ppm) zirconium was added to the UBi. In the dynamic tests the fuel is circulated either by convection or by means of a pump through piping of the above steels in which the fluid is subjected to a temperature differential. This method of testing simulates more realistically the conditions under which the materials will be used in a reactor circuit. Since the constituents of the materials have a positive temperature coefficient of solubility in the fuel, this test results in a more severe attack by the mechanism of mass transfer. In the hotter sections of a loop circuit, the materials in contact with the fluid dissolve in an attempt to come to equilibrium with it. The amount of material that dissolves as a given quantity of UBi flows through the hotter section depends on the rate of solution and the residence time in the hot region. When the hot liquid is cooled as it passes through the heat exchange section of the loop, the constituents that have dissolved in the liquid may reach saturation, and precipitation of the excess dissolved material occurs. Mass is thereby transferred from the hot to the colder regions of the loop. In one thermal convection loop test in which UBi was circulated through a temperature differential of 70° C. (550 to 480° C.) in a 0.622-inch inner diameter 2¼ per cent Cr steel pipe, flow stopped after 400 hours due to the deposition of iron and chromium in the colder section of the loop. The solubilities of iron and chromium at 550° C. are 28 and 85 ppm, respectively.

Methods for reducing mass transfer of steels by UBi have been thoroughly studied in both static and dynamic systems. It has been found that additions of zirconium (250 ppm) and magnesium (350 ppm) to the UBi reduce the mass transfer very markedly. For example, a loop run under conditions more severe than cited above except for the zirconium and magnesium additions had not plugged after 13,000 hours of operation. The conclusions that may be drawn* from having operated 4 pumped loops and 50 thermal convection loops to determine the importance of such variables as velocity, temperature differential, maximum temperature, types of steels, effect of heat treatment of steels, and the various inhibitors Zr, Ti, Mg, Th, Ca, are: (1) velocity up to 14 ft./sec. has little effect; (2) mass transfer rates in inhibited systems are low and are dependent on both the temperature differential and the upper temperature of operation; (3) carbon steels show less corrosion (mass transfer and pitting) than the chromium steels; increasing the Cr content increases the susceptibility to attack in inhibited (but not in noninhibited) systems; (4) a normalizing and tempering heat treatment yields a steel which is

*The first five conclusions were obtained with a UBi fuel that contained 1000 ppm of Uranium, 350 ppm of magnesium, and 250 ppm of zirconium.

less prone to pitting than an annealed steel; (5) a pit type of attack of low chromium steels by inhibited UBi usually is of a pitting nature, whereas attack by noninhibited UBi is principally intergranular; (6) zirconium is the best inhibitor tested; and (7) magnesium additions enhance the effectiveness of zirconium as an inhibitor, whereas calcium and thorium do not.

Mechanism of inhibition. From the results obtained in the corrosion tests it is obvious that if steels are to be used for containment of the fuel system an inhibitor such as zirconium must be used. Since there is limited experience in the use of inhibited liquid metal systems and because so much reliance must be placed on the inhibition, a research program to determine the mechanism of inhibition was undertaken. The results of this investigation may be summarized as follows: (1) zirconium (radioactive) dissolved in bismuth is adsorbed on pure iron and steel; (2) the amount adsorbed on pure iron depends on the zirconium concentration, the contact temperature, and the contact time; (3) zirconium in the amounts added does not reduce the solubility of iron and chromium in bismuth; (4) the rates of solution of the iron from the low chrome and carbon steels in bismuth are markedly reduced when zirconium and magnesium are present in the bismuth solution; (5) iron is present at 2 to 3 times its normal solubility in zirconium bismuth solutions that are cooled down from a higher temperature at which they were partially saturated with Fe; (6) X-ray diffraction studies have shown that a surface layer of a zirconium compound, ZrN or ZrC, forms on the steels contacted with UBi containing Zr + Mg; (7) these surface films have been identified on only a few low temperature-treated specimens (550° C.), but are readily found on the high temperature ones (700° C. and up); (8) the composition of the film, ZrC or ZrN, depends on the ratio of total nitrogen to soluble aluminum, and in some way on the amount of AlN present in the steel;* (9) the inhibition is therefore attributed to the formation and the perfection of these ZrN or ZrC films, which are insoluble in UBi; these reduce the rate of dissolution of steels by imposing a barrier through which the Fe or Cr must diffuse to enter the solution; and (10) these films can be stripped from the steels by a proper etchant, such as bromine dissolved in methanol. This technique permits a more thorough study of the films by chemical, X-ray diffraction, and electron diffraction analysis.

The effects of reactor radiations on the behavior of the fuel system and materials have not been presented because these experiments are still in progress and the information available is too meager to permit evaluations. In summary, we feel that, although many of the materials

*Nitrogen is usually considered an impurity in steels. In the manufacture of steels, aluminum is frequently added as a deoxidant. The excess Al, over that required to tie up oxygen, reacts with the nitrogen impurity to form AlN. The completeness of this reaction depends on the heat treatment of the steel.

problems of the LMFR remain to be solved, the results obtained to date warrant further research and development of this promising concept.

In this paper the details of experiments and the bases for arriving at some of the conclusions have been omitted. For more details, the reader is advised to consult the following articles and the recent BNL Progress Reports.

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THE NEW YORK ACADEMY OF SCIENCES

SECTION OF PSYCHOLOGY

SOME FACTORS INFLUENCING BEHAVIOR IN A TWO-RESPONSE SITUATION*

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In their recent work, Ferster and Skinner[1] refer to a group of procedures that they designated as "concurrent schedules." In one of these procedures an animal is given access to two response keys simultaneously. The consequences to the animal making either response are independently manipulable. The organisms they used for these studies were pigeons; the responses were the pecking of either of two hinged, Plexiglas disks that were mounted on a vertical plane in the experimental chamber. The two responses could not occur strictly simultaneously, since pecking at one key precluded pecking the other at that moment, but the opportunity for either response was continuously and concurrently present. The pecking behavior was maintained by occasionally giving the pigeons (which were hungry) access to food for a brief period of time as a consequence of a peck on one or the other of the two keys.

The experiments to be described here fall into this same area of research. Food-deprived pigeons are employed; pecking either of two keys are the responses; and the behavior is maintained by reinforcement with food. Several ends appear to be reasonably well served by this general procedure. First, because the two responses are incompatible with each other in the sense that they cannot occur at the same moment, there is the possibility of studying choice as a behavioral process. When the pigeon pecks at one of two available keys, it has, at a descriptive level at least, informed us of the resolution of two tendencies to respond at that moment. Second, closely related to the above is the investigation of how relative tendencies to engage in these two responses are influenced by experimental manipulations. In a sense, this question simply integrates over a number of individual choices between the two responses, and therefore would be more appropriately included in the study of choice. They are mentioned separately, however, because the study of choice demands incompatibility of the two responses, whereas the study of relative tendencies does not. Third, knowledge concerning the properties of behavior to a single response key in this type of experimental situation has reached a level sufficiently advanced to make

*This paper, illustrated with slides, was presented at a meeting of the Section on October 20, 1958.

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possible a fruitful evaluation of the complexities that arise from the addition of a second key.

Concurrent Ratio Schedules

In the schedule of intermittent reinforcement called "fixed ratio," an animal is reinforced for each *n*th occurrence of some selected sample of its behavior. For example, a pigeon may be trained to peck at a disk and then be given access to food for 4 sec. as a consequence of each fiftieth peck. This schedule of reinforcement would be specified as a "fixed ratio of 50," or FR(50).

An experiment was performed with pigeons, in which each of two response keys was associated with a particular value of fixed-ratio schedule. The two keys functioned independently; that is, the completion of the ratio on one key had no effect on the status of the other. When the ratio values on the two keys differ by factors of 3 or 4 to 1 [e.g., FR(200) on one key; FR(50) on the other], virtually all of the pecking behavior is restricted to the key associated with the lower value. The tendency to peck at only one key does not, however, disappear when the values associated with each key are set equal to each other. It might be added that the selection of a preferred key, in the case of equal ratio values, is not obviously related to any of the external properties of the situation (such as minimal force of response, location, or visual characteristics of the two keys), nor to the prior experience of the animals.

The first six sessions plotted in FIGURE 1 show, for two pigeons, what proportion of all responses are made to one key when the schedule of reinforcement is FR(210) on each key. One pigeon pecks exclusively on key A; the other has a less marked preference, but for key B. These preferences are unstable, showing varying degrees of completeness at different times and, occasionally, shifting from one key to the other. Even with several hundred hours of exposure to one set of ratio values, the preference does not, however, give any indication of actually disappearing.

Without entering into a detailed analysis of this finding, it can be stated that the predominance of responding to one key probably results from two characteristics of the experimental situation. First, the likelihood of receiving reinforcement on one key is independent of the number of responses emitted to, or the duration of time spent at, the other key. Second, the prepotence of one response is probably favored by using responses that are topographically incompatible with each other, and the animal therefore must engage in some form of explicit "switching" behavior in order to change its response. Presumably, appropriate variations in any of these characteristics would result in a reduction in the degree of prepotence.

A concurrent ratio schedule was investigated in which the occurrence of reinforcement on one key depended upon responses to the other.

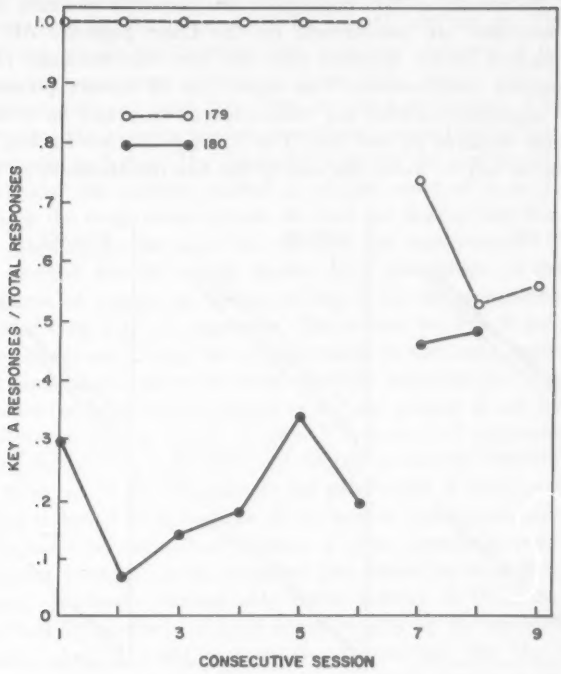

FIGURE 1. The first six sessions show the proportion of total responses that are emitted to key A when the two keys have equal, but independent, likelihoods of reinforcement.

Pigeons are reinforced with food for the emission of at least 40 responses. Of the 40, at least 20 responses must be made to one key, and at least 20 to the other. The response that is reinforced may be to either key. For example, if the key-A requirement (that is, 20 responses) is met first, then, in this particular instance, a key-B response is reinforced. In all cases, the reinforcement will be delivered for a response to the key on which the requirement is met last.

The response requirements for each key are varied while the total requirement (the sum of the individual requirements) is held constant at 40 responses. The key-A and key-B requirements are, respectively, 20 and 20; 10 and 30; 30 and 10; 35 and 5; and 2 and 38. Two pigeons were run through this series in the above-mentioned order. A third pigeon was studied at three sets of values only: 30 and 10; 35 and 5; and 2 and 38. Each set of values was maintained until the responding ceased to show any progressive changes. Thirty experimental sessions, each consisting of 60 reinforced sequences, generally sufficed.

Some of the results of this experiment are shown in FIGURE 2. Four different measures are summarized for the three pigeons. All points represent median values obtained from the last five sessions for each set of response requirements. The upper row of curves presents the number of responses emitted per reinforcement on a key as a function of the number required on that key. The filled points are for key A; the open points for key B. Since the sum of the two requirements is always

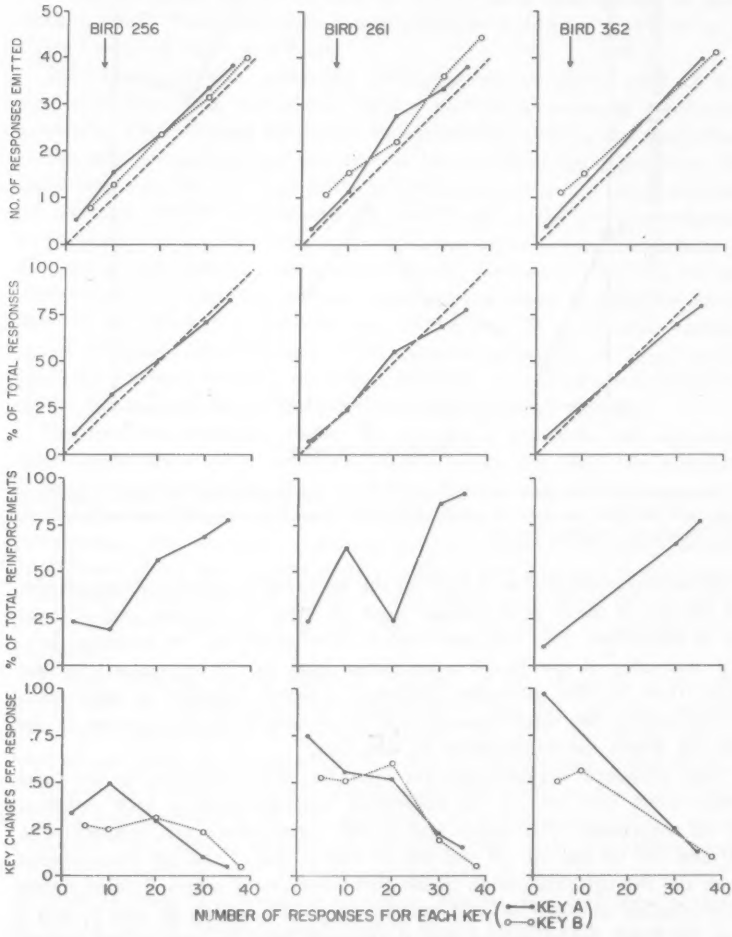

FIGURE 2. A summary of the results of making the consequences of responding to the two keys noninderpendent (see text).

40, the point plotted at a requirement of 2 for key A was obtained at the same time as the point at 38 for key B, and so on. The diagonal dashed line shows the function that would be obtained if the behavior matched the requirement exactly; that is, if the pigeon were to complete the requirements on each key with no extra responses. These curves indicate that the pigeons are being precisely controlled by the particular requirements in force. The disparity between the number of responses required and the number emitted is of the order of 3 to 7 responses throughout the range investigated. It does not appear that the magnitude of the disparity varies with the value of the requirement.

The second row of curves shows what percentage of the animal's total output of responses occurs to key A as the requirements on this key varied from 2 to 35 responses. The curves for key B are not shown because they are simply the complements of the ones presented. The dashed lines again show the most suitable performance. There appears to be some tendency for the output to deviate upward at low requirements and downward at the higher values. The extent of agreement between the obtained and optimal functions is, nevertheless, surprisingly great.

The third row of graphs presents the percentage of total reinforcements occurring to key A as a function of the size of the requirement on key A. Perhaps the most significant feature of these curves is that no orderly and regular relation seems apparent (the curve for bird 362, it should be noted, is drawn through only three points). If these curves were invariant at 50 per cent, or rose monotonically as the requirements were increased, then it might reasonably be supposed that the adjustment of behavior to requirement shown in the upper functions is based on adjustments in reinforcement frequency. The obtained relation does suggest that the reinforcement frequency increases with the size of the requirement, but the relation is distinctly erratic. If reinforcement frequency were in fact, critical, then one might expect the deviations in this relation to be reflected in substantial deviations in the functions presented in the upper two rows of FIGURE 2. Those functions, however, appear to be highly stable.

The curves in the third row are based on medians of five sessions. Under a given set of response requirements, the range of session-to-session variation in the percentage of reinforcements on a given key was as much as 50 per cent in several instances. Medians based on 13 sessions instead of 5 still show irregularities, although the case for a monotonically increasing relation is somewhat strengthened. A more direct evaluation of the importance of reinforcement frequency was attempted in an experiment that modifies the original procedure. Reinforcement is still dependent upon the emission of a minimum number of responses to each key, but the experimenter explicitly governs whether a key-A response or a key-B response is reinforced. The requirements were held at 2 and 38 responses for key A and key B, respectively.

Under one procedure, only key-A responses are reinforced. If the key-B requirement is attained last, then the animal must return to key A for one more response in order to be reinforced. Thus, the new procedure interacts with the size of the requirement by occasionally demanding a minimum of 3 responses, instead of 2, to key A. Since the average number of responses to key A was greater than 3 in the earlier experiment, this intermittent change in the size of the requirement was not expected to be, in and of itself, significant. A second procedure maintains the size of the requirements at 2 and 38, but reinforces only key-B responses. A similar interaction now occasionally demands 39 responses to key B. The 2 procedures were investigated for 20 and 8 experimental sessions, respectively, that is, until the behavior appeared to become stable under each condition.

The results of this experiment, for 3 pigeons, are presented in FIGURE 3. The medians of the terminal 5 sessions for each procedure are shown.

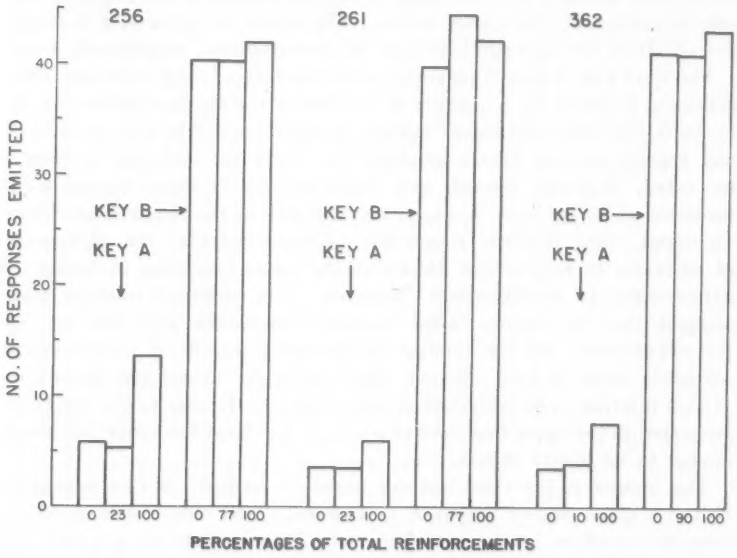

FIGURE 3. Responding to the two keys with response requirements held constant, but reinforcement frequency varied. Performances for three pigeons are shown.

The ordinate shows the average number of responses that were emitted on a key in a single reinforced sequence. The abscissa shows the percentage of reinforcements received on that key. There are 2 groups of 3 bars for each pigeon, 1 group for each key. The center bar of each group of 3 is taken from the results of the earlier experiment, in which

reinforcement frequency was not being directly controlled. The bar at zero per cent for key A was obtained at the same time as the bar at 100 per cent for key B, and vice versa.

The most prominent feature of these data is that the deliberate control of reinforcement frequency does not counteract the control exerted by the response requirements on each key. Key-B responding remains substantially above key-A responding when only the latter responses can be reinforced, and reinforcing only the former does not markedly reduce the level of key-A responding. However, there is some effect of altering reinforcement frequency. This is most apparent in the consistent increase obtained in key-A responding when only key-A responses are reinforced. The corresponding effect is less pronounced, but probably present, when key-B responses are being reinforced. For neither key A nor key B is there a consistent decrease in responding when these keys are never reinforced.

The exact sequence of responses to the two keys was not recorded. The bottom row of FIGURE 2 does, however, present some information concerning the manner in which switching from one key to the other is affected by the size of the response requirements. The values on the ordinate are obtained by dividing the number of times a switch is made from a given key to the other by the total number of responses on the given key. It is a measure, therefore, of the probability of a switch away from a given key. The filled points are for switches from key A to key B, and the open points for switches in the other direction. There is, in general, a decrease in the probability of a switch away from a key as the requirement on that key is increased. This finding is perhaps more readily interpretable if one considers the reciprocal of the ordinate: the average number of responses on a key that occurs between a switch *to* the key and the switch *away* from it. As the requirement is increased there are, roughly speaking, longer series of responses on a key that are uninterrupted by a switch to the other key. It is noteworthy, however, that this relation is only a rough one. The tendency for the probability of a switch to decrease becomes clearly evident only at the higher values of the requirement. With lower values (up to around 20), the probability does not change, or does so only slightly. The only curve that shows a steady decline is the one that has no intermediate points between requirements of 2 and 30 (bird 362, key A). Thus, although the length of a run of responses on a key increases with the size of the requirement, it is unlikely that this is the primary mechanism by which the adjustment of behavior to requirement is mediated.

The degree to which one response is prepotent is reflected in the absolute frequency with which switches between the two responses occur. FIGURE 4 presents this information for the three pigeons. The total number of key changes per session (based on medians of five sessions) is plotted as a function of the absolute size of the difference

between the requirements on each key. Prepotence was previously shown to become more pronounced as this difference was made larger. In FIGURE 4, it can be seen that the greatest amount of oscillation between keys is obtained when the requirements are equal, and that the amount decreases proportionally as the difference between requirements is increased.

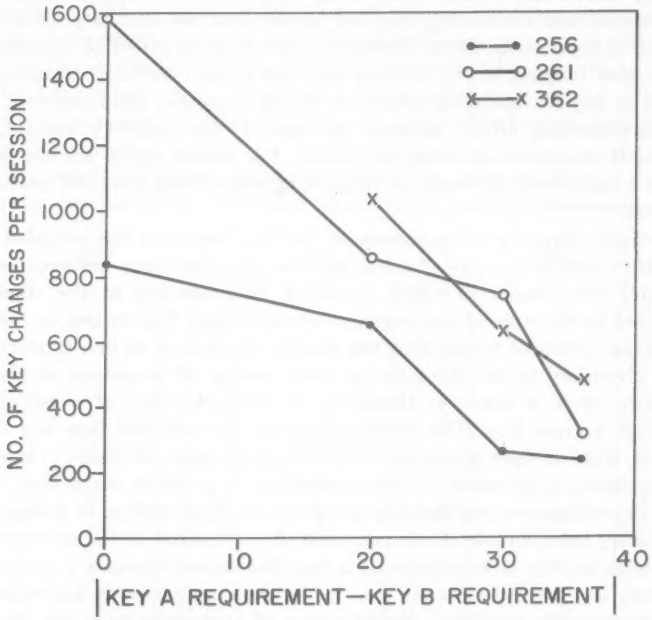

FIGURE 4. The amount of oscillation between the two keys as a function of the absolute difference between the response requirement on one key and that on the other. Each curve is for one pigeon.

FIGURES 5, 6, and 7 show, for each pigeon, representative cumulative-response curves obtained under all the values of the response requirements that were summarized in FIGURE 2. In these three figures, the key-A records are brought to one common origin and the key-B records are brought to another in order to facilitate visual inspection of certain features of the performance. Each single curve shows the performance on a particular key for an entire session. The curve labeled *1* for key A is concurrent with the one labeled *1* for key B, and so on. The two recording devices ran continuously and concurrently during experimental sessions; each recorder, however, cumulated responses to one key only. The slopes of these curves are therefore proportional to the absolute rates of responding on a particular key. The small, diagonal marks on

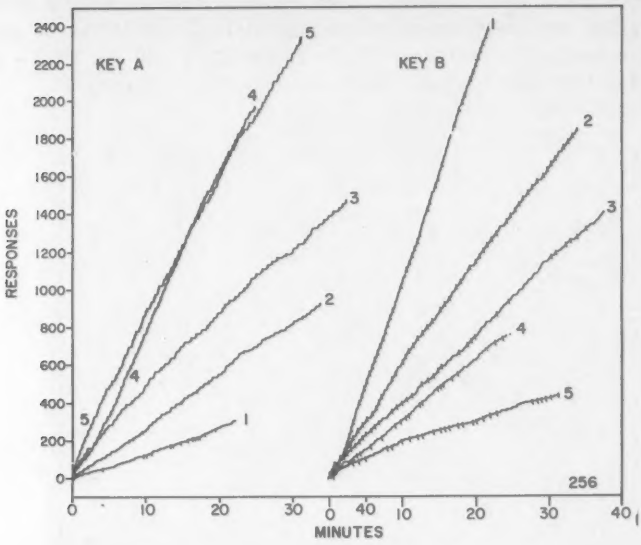

FIGURE 5. Typical cumulative-response curves obtained from one pigeon, showing the performances on each key with the various response requirements (see text).

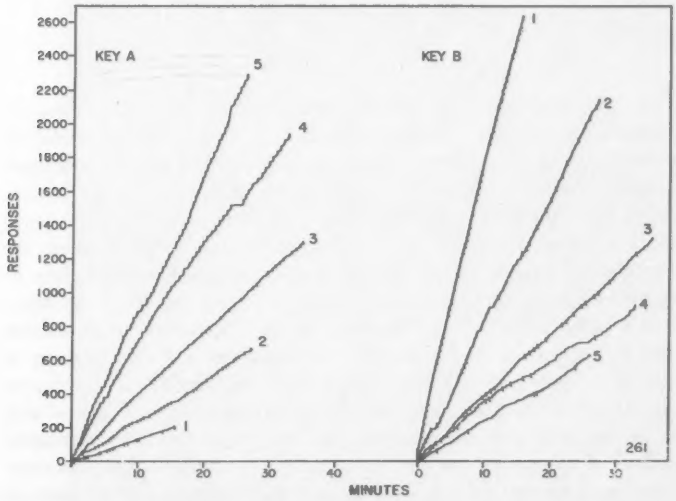

FIGURE 6. Same as FIGURE 5, for the second pigeon.

the curves signal reinforcements. The response requirements for birds 256 and 261 were as follows: curves labeled *1* – 2 and 38 (key A and key B, respectively); *2* – 10 and 30; *3* – 20 and 20; *4* – 30 and 10; *5* – 35 and 5. For bird 362: *1* – 2 and 38; *2* – 30 and 10; *3* – 35 and 5.

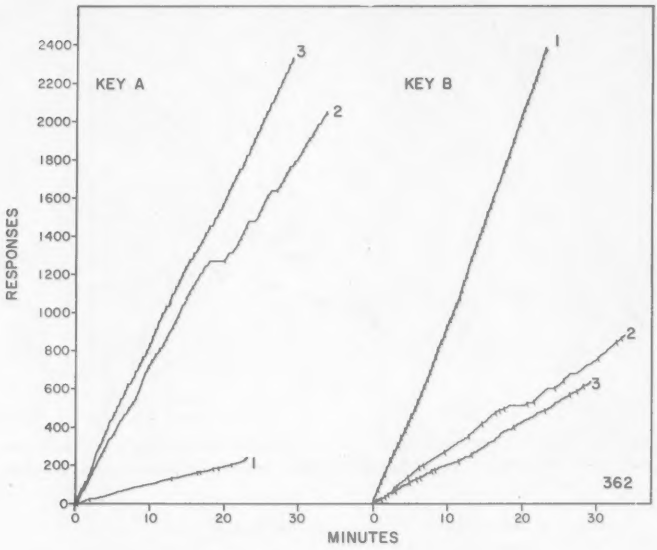

FIGURE 7. Same as FIGURE 5, for the third pigeon.

Several characteristics of these curves are of particular interest. In all cases but one (key A, curves *4* and *5*, bird 256), the absolute rate of responding is related directly to the size of the requirement. The larger the requirement, the higher the slope of the cumulative record. In addition, the duration of the experimental session, which is determined by the time taken to obtain 60 reinforcements, is longest when the requirements on two keys are equal, and is progressively shorter as the requirements are made more dissimilar. The one exception is again at curves *4* and *5* for bird 256. It is possible that this decrease in session duration is related to the decrease in switching behavior shown in FIGURE 4. Finally, it might be noted that the heights of the curves (that is, the total number of responses in a session on a given key) are in accord with the requirements. As the requirement is increased, the number of responses emitted in a session increases. This relationship was summarized in FIGURE 2, but the present figures indicate that the relationship is evident within single sessions.

Summary

A two-response situation involving independent fixed-ratio schedules of reinforcement on each key was investigated. It was shown that, even when the likelihood of reinforcement is equal for both responses, pigeons show predispositions toward one response or the other. The experimental procedure was modified in such a way that reinforcement of responding on one key was no longer independent of responding to the other. In this procedure, reinforcement was conditional upon the emission of a minimum number of responses to each key. It was found that this technique put the dominance of one response or the other under the experimenter's control. Several intermediate degrees of dominance were obtained by manipulating the number of responses required to each key.

Acknowledgment

Gratitude is expressed to W. H. Morse, Harvard Medical School, with whom I collaborated on the first study and who made many helpful suggestions concerning this article.

Reference

1. FERSTER, C. B. & B. F. SKINNER. 1957. Schedules of Reinforcement. Appleton-Century-Crofts. New York, N. Y.

DIVISION OF MYCOLOGY

THE SAPROPHYTIC PHASE OF THE FUNGI OF CHROMOBLASTOMYCOSIS: EFFECT OF NUTRIENTS AND TEMPERATURE UPON GROWTH AND MORPHOLOGY*

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Although much is known about the clinical, histopathologic, and epidemiologic aspects of chromoblastomycosis and about the morphology and immunology of the etiological fungi,[9] the taxonomy of these fungi is still not generally agreed upon,[4,10,12] and the disease remains a therapeutic problem. Only in small, comparatively early lesions have surgery or chemotherapy[5,14] proved effective.

In the hope that information on the physiology of these fungi might help solve the problems they created, various aspects of their behavior were studied.[20] A portion of these studies dealt with the parasitic phase and consisted of an attempt to obtain regularly, and in quantity, a supply of the sclerotic cells that characterize it.[19] Another portion dealt with the saprophytic phase and will be presented here in part.

Since this report deals with the effect of nutrients on sporulation, a brief description of sporulation in the fungi of chromoblastomycosis is in order. These pathogens belong in the family Dematiaceae of the Fungi Imperfecti and are therefore identified by their conidia, which they produce by at least three different methods. Two methods are characteristic of each of two well-known genera: *Cladosporium (Hormodendrum),* producing conidia terminally in basifugal branching chains; and *Phialophora,* producing clusters of conidia pinched-off successively through an ostiole on the meristematic cell. In the third method, somewhat resembling that of *Acrotheca,* conidia are produced acropleurogenously, that is, on the tip and along the sides of a verrucous fertile cell. In addition to the uniseriate, terminal arrangement of spores, as in *Acrotheca,* secondary spores may appear, or the verrucous mother cell may be lateral or intercallary. Consequently, in the fungi of chromoblastomycosis this type of sporulation is designated the pseudo-*Acrotheca* (or *Fonsecaea*) type.

In addition to the studies on sporulation, the effect of nutrients and of temperature upon the growth of representative isolates will be discussed.

*This paper, illustrated with slides, was presented at a meeting of the Division of Mycology on October 24, 1958.

This article is a portion of a thesis accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Radcliffe College (Harvard University), Cambridge, Mass.

Materials and Methods

Fungi. TABLE 1 shows the taxonomic and geographical distribution of the isolates. Nineteen of them were the "basic selection" employed in studies of the parasitic phase;[19] four others were added where indicated for comparison. Three of these, identified as *Cladosporium carrionii*, were obtained through the courtesy of C. W. Emmons from Australian patients of E. H. Derrick; the fourth was a saprophytic strain of *Cladosporium* (air contaminant).

TABLE 1

CLASSIFICATION AND ORIGIN OF ISOLATES*

Classification	*No. of isolates*	*Sources*
I. *Fonsecaea pedrosoi*		
(a) *F. pedrosoi* var. *cladosporioides*	1	Algiers
(b) *F. pedrosoi* var. *communis*	6	5 from Puerto Rico 1 from Brazil
(c) *F. pedrosoi* var. *pedrosoi*	4	1 from Argentina 2 from Brazil 1 from South Africa
(d) *F. pedrosoi* var. *phialophorica*	1	Brazil
II. *Fonsecaea compacta*	2	1 from Puerto Rico 1 from Tennessee
III. *Phialophora verrucosa*	5	1 from Uruguay 5 from the United States (Boston, Mass.; Texas; St. Louis, Mo.; Philadelphia, Pa.)
IV. *Cladosporium carrionii*	3	Australia
V. *Cladosporium sp.* (air contaminant)	1	United States (Boston)

*Nomenclature and taxonomic treatment as proposed by Carrión[7,8] and Trejos.[23]

Media. Except for studies of nutritional requirements, the culture medium employed was Sabouraud's glucose agar prepared to contain 4 per cent C. P. glucose, 1 per cent Bacto peptone, and 2 per cent powdered agar in distiiled water. For determining the effect of culture media on sporulation, corn-meal infusion agar* and Czapek-Dox agar[22] were used, individually or combined in equal parts. For studies of the effect of carbon and nitrogen sources on growth and sporulation, modifications were made as indicated on the carbon and nitrogen source of the basic Czapek-Dox formula (FIGURE 1).

Methods. For studying sporulation, the fungi were grown on the customary slide cultures with a thin layer of nutrient agar spread over standard

*Prepared according to Benham's formula[1] from Quaker unenriched white or, where indicated, vitamin-enriched yellow corn meal, Quaker Oats Co., Chicago, Ill.

microscope slides in moist chambers at 30° C. for 3 weeks. This incubation period allowed full expression of the various types of sporulation, yet avoided the appearance of the bizarre morphologic changes sometimes occurring in aging cultures. By making differential counts of 100 conidiophores selected at random from widely scattered fields in a mature slide culture, a representative sampling of the entire "crop" of conidiophores was obtained. Duplicate slides were counted; the results between duplicates agreed within a very small range of variation.

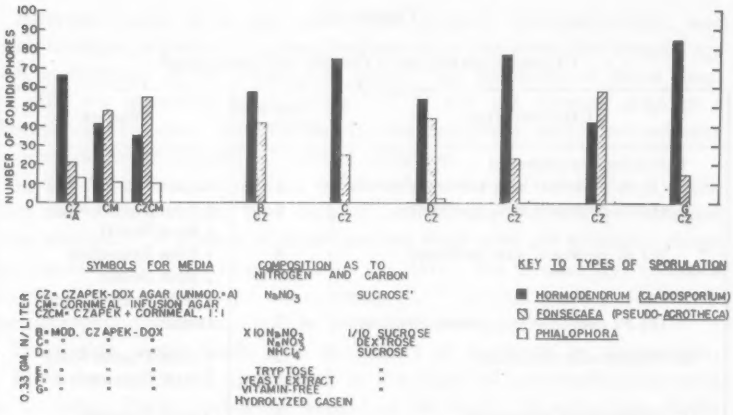

FIGURE 1. Influence of nitrogen source on frequency of conidiophore types in one representative isolate: *Fonsecaea pedrosoi* var. *communis* (S-399Q).

To measure growth, the technique employed by Ryan *et al.*[18] was chosen. By constraining the fungus to grow within sections of glass tubing held horizontally and half-filled with agar media, the mycelium advanced in one direction and its progress was measured linearly along the long axis of the tube. Two types and sizes of "growth tube" were used: (1) Ryan's, consisting of long sections of 25 mm.-wide glass tubing, cotton-plugged and bent at both ends so as to leave a straight 25 cm.-long horizontal section for the agar; and (2) a smaller one, made from ordinary test tubes, 120 × 12 mm., dented across one side of the wall near the lip by pressing the heat-softened tube against a metal rod so as to leave a 1 × 10 cm. trough for the agar. The longer tube served for measuring growth of the fungi on Sabouraud's agar at room temperature; the shorter one for testing media at temperatures markedly reducing the rate of growth. To prevent drying of the thin films of media in the growth tubes when incubated for long periods or at high temperatures, a tight seal of Parafilm* was applied over the cotton plugs. To prevent growth of

*Product of Marathon Corp., Menasha, Wis.

mold contaminants under this seal, the Parafilm was sterilized by immersing it in 70 per cent alcohol for 30 min. or more and then draining it between sheets of sterile paper towels. The cotton plugs were flamed and cooled just before sealing.

In all cases, duplicate tubes were measured and, as indicated, they were incubated either at uncontrolled room temperature (approximately 27° C.) or in a series of controlled-temperature chambers.

Results

Sporulation on Czapek-Dox and corn-meal agars. The long-established superiority of these two media over Sabouraud's agars for inducing sporulation in these forms, plus the previous observation[7,9] that, in *F. pedrosoi*, Czapek-Dox favored the production of conidiophores of the *Cladosporium* type and corn-meal infusion agar the pseudo-*Acrotheca*, raised two questions: (1) Which constituents in corn-meal and in Czapek-Dox agars exerted these effects? (2) Would the subspecies of *pedrosoi* remain true to type when given substrates that might alter the proportions of the sporulating types?

To confirm the previously observed effect on sporulation of these two media on a firmer quantitative basis, and also to scrutinize isolates that, because of lack of one or another sporulating form had been classified in genera exhibiting only one type of sporulation, the twenty-three isolates were grown in slide cultures on each of these media, individually and on a mixture of equal parts of the two. After three weeks' incubation the differential counts (FIGURE 2) showed or confirmed the following:

Phialophora verrucosa produced only one kind of sporulation, the *Phialophora* type, on all three media; similarly, only one method of sporulation, the *Cladosporium* type, was produced by the three isolates of *C. carrionii*. The saprophytic strain examined of this same genus produced practically all its conidia in typical acrogenous *Cladosporium* fashion; the only suggestion of an acropleurogenous arrangement was given by the occasional transformation of a spore, still in a *Cladosporium* head, into a warty conidiophore. This was observed in only three instances, once on a corn-meal slide, twice on a corn-meal-Czapek slide.

In contrast to these two types, isolates classified as *Fonsecaea pedrosoi* sporulated by all three methods, although in varying degrees. In members of the subspecies *communis* all three methods were produced readily, but in all of them the proportionate representation of conidiophores of pseudo-*Acrotheca (Fonsecaea)* type was highest when corn-meal agar was supplied, whether undiluted or reduced to half strength by an equal part of Czapek-Dox agar. This type of sporulation was also more abundant on the same media in the subspecies *pedrosoi (typica)*, and in the subspecies *cladosoporioides*, but not in *phialophorica*. *Fonsecaea compacta*, on the other hand, behaved in opposite fashion; that

is, the acropleurogenous arrangement of spores (pseudo-*Acrotheca* type) was considerably diminished, not increased, on corn-meal agar.

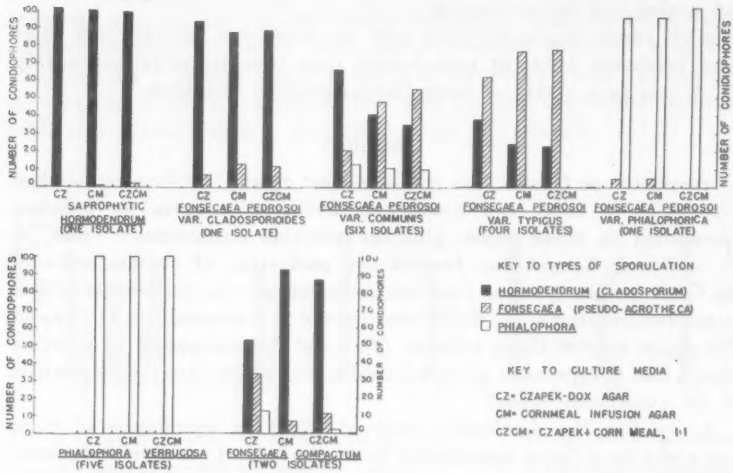

FIGURE 2. Influence of culture media on frequency of conidiophore types in isolates representative of the various taxa examined.

What constituent in corn-meal agar that is absent in Czapek-Dox increases the pseudo-*Acrotheca* type of sporulation in the species *pedrosoi*? Since the main attribute of corn-meal agar is its organic nutrient content, it seemed important to determine whether incorporating richer sources of nitrogen – inorganic and organic – and changing the carbon source in the basal formula for Czapek-Dox agar would stimulate the production of pseudo-*Acrotheca* conidiophores in some isolates.

Sporulation on various carbon and nitrogen sources. In an attempt to answer the question just formulated, the nutrients and media listed in the section on methods were studied for their effects on four isolates selected from the original nineteen because the medium influenced their sporulation more conspicuously than the others. The response of one representative isolate is shown in FIGURE 1. The frequency of one or another type of conidiophore on these media, when compared with that in the unmodified Czapek-Dox formula, permits the following observations:

Inversion of the C:N ratio in Czapek-Dox agar (Medium B, sucrose decreased to 1/10, $NaNO_3$ increased tenfold) increased the frequency of the pseudo-*Acrotheca* type of sporulation.

Substitution of glucose for sucrose (Medium C) did not significantly alter the *Cladosporium*:pseudo-*Acrotheca* ratio.

Use of NH_4Cl in place of $NaNO_3$ (Medium D) increased the frequency of the pseudo-*Acrotheca* type of sporulation.

Substitution of Tryptose for $NaNO_3$ (Medium E) did not change the *Cladosporium*:pseudo-*Acrotheca* ratio.

Incorporation of yeast extract in the medium (Medium F) greatly increased the proportion of pseudo-*Acrotheca* conidiophores.

Use of vitamin-free casein hydrolysate instead of yeast extract (Medium G) did not increase the production of pseudo-*Acrotheca* conidiophores over that in the basal medium (unmodified Czapek-Dox).

The proportion of pseudo-*Acrotheca* conidiophores was even greater on yellow corn-meal agar than on white corn meal.

Growth on various carbon and nitrogen sources. After preliminary determination of the growth rates of the original 19 isolates on Sabouraud's glucose agar at room temperature, 4 isolates were selected for further study: a fast-growing one (No.S-399H, 10 mm./wk.), a moderate one (S-399D, 4.6 mm./wk.), and 2 slow ones (S-399Q, 1 mm./wk. and S-399E, 0.7 mm./wk.).

First, the effects of inorganic versus organic nitrogen and of glucose versus sucrose were determined by growing them for 3 weeks at 30° C. on the same media used to study sporulation. By means of a bar diagram, FIGURE 3 shows the rates of growth obtained on these media and on Sabouraud's glucose agar. From these data it becomes evident that inorganic nitrogen (Media A to D) is as suitable as organic (Media E to F) for the growth of these organisms (see also Montemayor[16]). Growth was least and slowest for all 4 isolates on Medium B, which contained only one tenth the amount of carbohydrate present in the other media. Since Medium B also contained at least 10 times as much nitrogen as any of the other media, it may be concluded that the amount of carbohydrate, rather than the amount of nitrogen, was the factor that influenced the rate of growth.

To investigate a wider range of carbon sources, the same 4 isolates were grown on 12 different carbohydrates, chosen to include pentoses, hexoses, disaccharides, and polysaccharides. Eleven of the carbohydrates, including 9 C.P. grade chemicals: glucose, fructose, xylose, both D- and L-arabinose, sucrose, maltose, lactose, and cellobiose, and 2 starches: soluble starch and corn starch, were incorporated in the Czapek-Dox formula in a 1 per cent concentration. The twelfth, cellulose, was incorporated as macerated filter paper (0.5 per cent). The inocula for these media were small fragments of aerial growth taken as usual from colonies on Sabouraud's glucose agar, but here they were repeatedly washed in sterile distilled water to avoid carrying over glucose from the substrate of the parent culture. Incubation again was at 30° C. The results on these carbohydrates are illustrated in a bar graph in FIGURE 4. As shown, all monosaccharides tested: glucose, fructose, xylose, L- and D-arabinose, supported growth of the 4 isolates

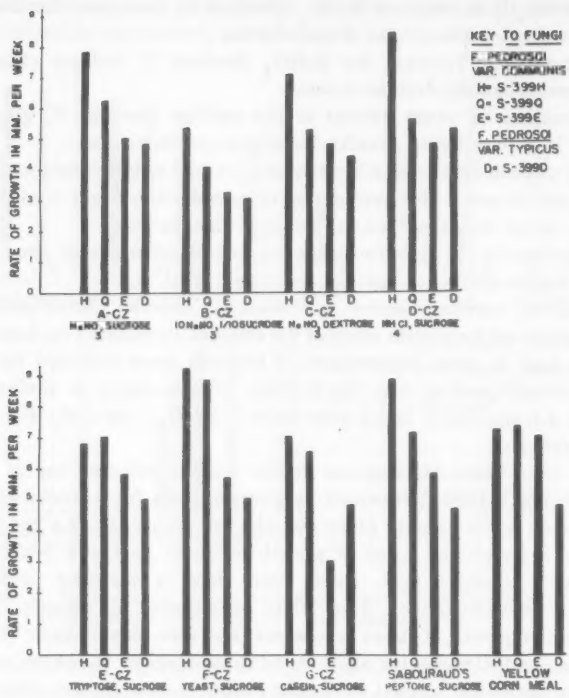

FIGURE 3. Influence of nitrogen and carbon source on rates of growth of four selected isolates (grouped according to substrate). Note that growth was slowest, for all four isolates, on Medium B, containing one tenth the amount of carbohydrate in the other media.

well, and at comparable rates. The same was true of the disaccharides sucrose and maltose, but not of lactose, which supported somewhat less growth at a slower rate, nor of cellobiose, which supported only meager and extremely slow growth. Of the polysaccharides, soluble starch and corn starch also were utilized to advantage, supporting good growth rates; growth on cellulose was poorest of all. In fact, growth was so poor on this substrate that there was doubt as to whether the scant growth resulted actually from the breakdown of cellulose or from traces of nutrient carried over with the inoculum.

Growth and survival at various temperatures. To determine the cardinal temperatures affecting linear growth, the same 4 isolates selected for the study on nutrients were employed. Growth rates were determined from duplicate modified growth-tubes of each organism incubated for 3 weeks at 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50° C. These determinations are summarized in FIGURE 5.

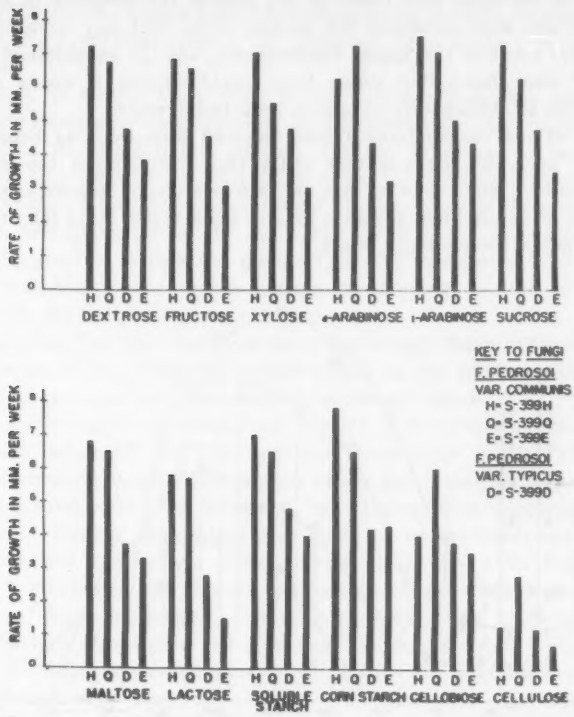

FIGURE 4. Influence of various carbohydrates on rates of growth of four selected isolates (grouped according to substrate). Note meager growth occurring on cellulose (see text).

As seen, no isolate grew at 5° C., but at 10° 3 grew to some extent, but slowly. The fourth isolate grew at 15° C.; thereafter the slopes of the growth rate curves for all 4 isolates rose steadily, though gently, to the highest, that is, optimum. This lay in a plateau extending from 25 to 30° C. for the slowest-growing isolate (S-399D), but was sharply 30° C. for isolates S-399H and S-399E, and 35° C. for isolate S-399Q. Above the optimum temperatures the growth rate declined rather sharply to zero; none grew at or above 40° C.

When cultures not showing growth after 3 weeks were returned to room temperature, growth was resumed from inocula that had been at 5 and 10° C. but not at 40, 45, or 50° C. Exposure for 3 weeks at the higher temperatures had evidently killed all 4 isolates.

That this lethal effect was due to temperature and not to reduced oxygen tension or desiccation was concluded from the following: (1)

sealing of the tubes with Parafilm had insured the retention of adequate moisture and also prevented the escape of air that may have expanded inside the tubes at the higher temperatures; and (2) unpublished experiments[20] had shown that these fungi could survive 3 weeks of total deprivation of atmospheric oxygen at room temperature.

None of the temperatures tested induced formation, on Sabouraud's glucose agar, of the sclerotic cells that characterize the parasitic phase of these fungi, showing that, unlike *Blastomyces dermatitidis*,[11,13,17] the fungi of chromoblastomycosis require some factor other than temperature to induce the parasitic phase.

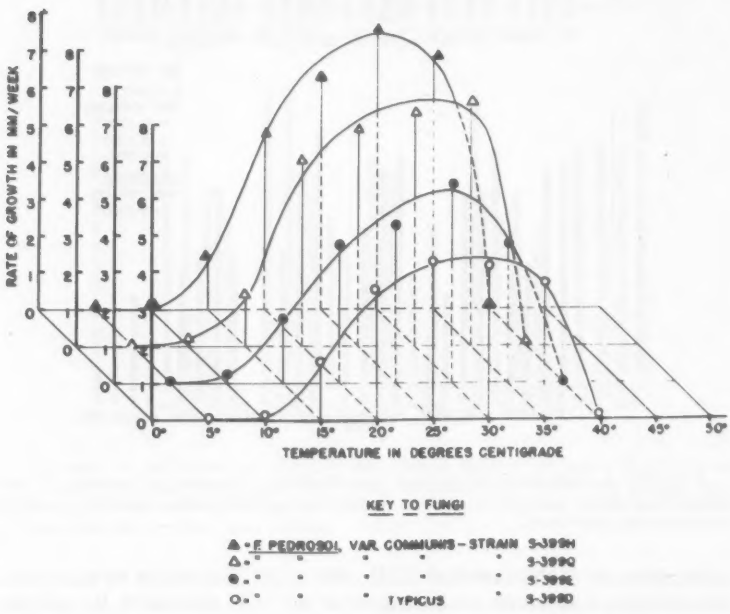

FIGURE 5. Influence of temperature on the rate of growth of four selected isolates. Note absence of growth below 10° or above 40° C.; optimum *not* at 37° C.

Discussion

From the studies on sporulation, it appears well established that most of the fungi of chromoblastomycosis fall, on stable morphologic grounds, into three major groups: one with sporulation exclusively of the *Phialophora* type; another with the *Cladosporium* type; and a third, in which these two types of sporulation may be represented together

with the pseudo-*Acrotheca* type. Classification of such triple-sporulating isolates in a separate genus, as proposed by Carrión,[7] is therefore justified.

Although it seems clear that, in the species *pedrosoi,* production of pseudo-*Acrotheca* conidiophores is influenced by the concentration and source of nitrogen and perhaps also by vitamins, no substance was found that consistently stimulated either the *Cladosporium* or *Phialophora* types or altered the proportionate representation of the sporulating types in any given isolate to the extent of requiring a change in its taxonomic position within the group. Thus the sporulating pattern, although variable, seems stable enough for a given isolate to justify subdivision of the species into varieties.

Recognition that this variation can exist among these pathogens and a knowledge of the effects of culture media on the relative proportions of conidiophore types are prerequisites to classification.

Some of the results on temperature deserve further comment. No growth took place below 10° C. This minimum temperature is high compared with that required by most filamentous fungi, and is particularly striking when compared with that tolerated by *Cladosporium herbarum.* This saprophyte, closely resembling the fungi of chromoblastomycosis in sporulation and morphology, grows slowly at -6° and even slightly at -10° C.[3,6] Whether ability to grow at this low temperature distinguishes pathogenic from saprophytic *Cladosporia* remains to be determined.

The optimum temperature for growth of these pathogens lay between 25 and 35° C., a range closely coinciding with the fluctuation reported for the human skin: from 23 to 34° C.[2] The maximum temperature for growth lay between 35 and 40° C. Since the temperature of the inner tissues and organs of the human body is somewhat higher than 37° C.,[2] temperature may be a factor barring their invasion of deeper tissues. An unexplained exception is the recent report of brain invasion by *Fonsecaea pedrosoi.*[15] The nonsurvival of these pathogens at temperatures above 40° C. suggests a possible method of therapy by artificially raising the temperature of the infected area. A 3° rise in temperature could be attained by either dry or wet heat and deserves clinical trial.

Summary

A study of the effect of culture media on the proportionate representation of the three types of conidiophore in the fungi of chromoblastomycosis was presented. Carrión's observation that corn-meal agar stimulates the pseudo-*Acrotheca,* and Czapek-Dox the *Cladosporium (Hormodendrum)* types of sporulation in *Fonsecaea pedrosoi* was confirmed. Combination of equal parts of corn-meal and Czapek-Dox agars affected sporulation as did corn-meal agar alone: that is, pseudo-*Acrotheca* conidiophores were significantly increased over those shown on Czapek-Dox agar alone by the same isolates. Increasing the concentration of inorganic

nitrogen in Czapek-Dox tenfold or adding yeast extract increased the proportionate representation of pseudo-*Acrotheca* conidiophores. Yellow corn meal was superior to unenriched white corn meal for the induction of this same type of sporulation. No substrate was found to induce isolates of *Phialophora verrucosa* or of *Cladosporium carrionii* to produce any other type of sporulation than the one characterizing their respective genera. Growth was also studied on the same media.

The cardinal temperatures for growth of these pathogens were determined: the minimum was between 10 and 15° C., the maximum, between 35 and 40° C.; the optimum, between 25 and 35° C., depending on the isolate. No growth occurred at or above 40° C., and the inocula were killed by a 3-week exposure to temperatures of 40, 45, and 50° C.

The possible application of these observations to the diagnosis and therapy of chromoblastomycosis are discussed.

Acknowledgments

I am indebted to William H. Weston, Jr. and Arturo L. Carrión for inspiring, supervising, and encouraging these studies, to Paul Pelech for rephotographing the thesis illustrations, and to Mildred Zurfluh for devoted secretarial help.

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SECTION OF ANTHROPOLOGY

A MECHANISM FOR MOBILIZING LABOR AMONG THE KUIKURU OF CENTRAL BRAZIL*

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Robert L. Carneiro
American Museum of Natural History, New York, N. Y.

In the normal course of events all societies are faced with certain tasks that need to be done and that require the cooperative effort of a large proportion of the society's labor force. The means by which this labor force is mobilized depends on the mechanisms available in a society for recruiting and organizing human effort. This paper deals with the mechanism for mobilizing group labor used among the Kuikuru Indians.

The Kuikuru, who comprise 145 persons, live in a single village at the headwaters of the Xingú River in central Brazil. The basis of their subsistence is slash-and-burn agriculture, with bitter manioc as their staple crop. Fishing provides an important dietary supplement, but hunting plays only a negligible role in the quest for food.

Unlike many horticultural groups, the Kuikuru are quite sedentary, having occupied the same locale for some ninety years. The village itself consists of nine large, multi-family thatched houses. Within each house live an average of four nuclear families. These nuclear families are often, but not always, related as extended families. There are no kinship units above the extended family: no lineages, sibs, or moieties. Thus, there are no unilineal kin groups through which the activities of large numbers of people might be organized.

Kuikuru political organization deserves particular attention, since it is through political means that in other societies labor is often mobilized. The Kuikuru recognize a chief who is the only political official in the community, there being no formal subchiefs or house chiefs. Very little authority is accorded to the chief, and his actual power varies with his personality. The present chief, Wafú, exercises almost no leadership. The recognized functions of a Kuikuru chief include exhorting people to work hard and to follow the traditional ways, representing the tribe in dealings with outsiders, and deciding when to move the village. We never heard Wafú exhort anyone and, when conditions arose that forced a consideration of whether or not the village should be moved, the decision fell to another man by default.

If we ask why chieftainship is so weak among the Kuikuru, the answer must be that the conditions that produce strong chiefs are absent. Hunt-

*This paper was presented at a meeting of the Section on October 27, 1958.

ing, which often requires strict adherence to a leader's orders, is all but lacking. More significant, however, is the absence of warfare. In a society where warfare is frequent and important, subordination to the orders of a recognized leader is imperative in time of conflict, and often it carries over into peaceful aspects of social life, as well. The Kuikuru do not engage in warfare and thus lack this stimulus to the development of strong centralized political control.

Very few activities in which the Kuikuru engage require energetic and forceful leadership. However, there are several that do require large-scale cooperative labor. These include housebuilding, the planting of fields on certain occasions, carrying canoes to the lake, and fishing with poison. In carrying out these activities some degree of coordination is of course necessary, but not a great deal.

In a society such as this, which lacks unilineal kin groups with strong leaders and political mechanisms for commanding the services of people, how is communal labor mobilized when needed? Among the Kuikuru the individual who initiates the enterprise also mobilizes the necessary labor.

When a man needs help in an undertaking he offers food and drink to those who will assist him. However, it is not only the prospect of food and drink that motivates people to participate. Work parties are festive occasions that are entered into in part for the conviviality they afford. Furthermore, there is probably the feeling that he who willingly helps others will be better able to find helpers when he himself needs them. For these reasons it is usually a simple matter to recruit a work party of twenty or thirty men.

The social mechanism for getting work done among the Kuikuru closely resembles their pattern of organizing ceremonies. In this pattern three sets of individuals respectively perform the roles of owners, askers, and singers. Each ceremony is owned by one or more individuals. An owner's permission must be obtained before his ceremony can be performed, and it is the owner who pays the bills, as it were, by furnishing food and drink for his festival. It is the askers' function to petition the owners to have their ceremonies performed. Singers are those who learn the songs and dances specific to a ceremony and who lead performances of them. Owners, askers, and singers all receive some recompense for carrying out their roles. The recompense to owners is primarily prestige and the appreciation of the society for their generosity; the askers and singers gain some prestige and, in addition, receive food presented ceremonially.

Some festivals consist entirely of ceremonial activities, but others incorporate work on projects that are not inherently related to ceremonialism. An example of the latter is the arrow ceremony held in conjunction with a corn harvest. A single individual, Tupá, owned both the arrow ceremony and the corn to be harvested. Otherwise there appeared to be no relation between the arrow ceremony and the corn harvest.

The festival began with a ritual in which the askers requested Tupá to permit his ceremony to be held. In exchange for food to be furnished by Tupá for the festival, the askers agreed to harvest his seed corn. The sequence of events was as follows. First a group of men performed songs and dances under the leadership of singers. Then the askers led most of the men in cutting timber that they dressed and carried to Tupá's house and set up as a frame on which to hang seed corn. Tupá's wife cooked manioc gruel, which she gave to the askers in payment for directing the work. A few days later they led a work party again, this time to harvest Tupá's seed corn. After the corn was delivered to Tupá's house there was more dancing. This time Tupá's wife prepared a large quantity of corn gruel and presented it to the askers who, in turn, shared it with the other men who had participated in the work and festivities.

This performance of the arrow ceremony combined two quite distinct functions. It provided a repertory of secular and recreational songs and dances, affording merriment for the entire community. In addition, it was also a means of mobilizing the cooperative labor required to reap a corn harvest and build a rack for storing it.

The two functions of entertainment and mobilization of labor are combined in a still more complex manner in some Kuikuru festivals. An example is the sacred flute festival. In this festival two sets of owners were involved. The flute ceremony itself is owned jointly by three men, while the flutes employed are owned by another man. On this occasion the owners of the ceremony petitioned the flute owner, Talá, to sponsor a festival, in return for which they agreed to construct fish traps for him. After obtaining Talá's consent, the askers led a work party to construct a weir across a nearby stream. For this the men of the work party received food and drink both from Talá and from the festival askers themselves in their capacity as owners of the ceremony. On the following day some of the men made basket traps to be set in the weir, while the rest of the men danced and sang. Both the men who made the traps and the dancers were recompensed with more food furnished by Talá. The traps were then ritually handed over to Talá, and for several days thereafter he gave the fish caught in them to the owner-askers of the festival.

Like the arrow festival, this one also had two distinct aspects. On the one hand it was an occasion for recreation and conviviality. On the other it served as a means for organizing and directing economically productive labor.

In summary, the distinctive features of the mobilization of labor among the Kuikuru are: (1) its degree of formalization; (2) its independence of kinship and political organization; and (3) its close integration with ceremonialism. We know of no system of mobilizing labor elsewhere in tropical South America that is comparable.

CONFERENCES HELD

SECTION OF BIOLOGY

CHLORPROPAMIDE AND DIABETES MELLITUS

Thursday, September 25,
Friday, September 26, and
Saturday, September 27, 1958

Conference Chairman: Martin Goldner
Jewish Chronic Disease Hospital, Brooklyn, N. Y.

THURSDAY, SEPTEMBER 25, 1958

PHARMACOLOGICAL STUDIES WITH CHLORPROPAMIDE

Session Chairman: Robert H. Williams
University of Washington, Seattle, Wash.

9:00 A.M. –
Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences; New York University, New York, N. Y.

"Preliminary Pharmacological Studies with Chlorpropamide" – Auguste Loubatiéres, Laboratory of Applied Physiology, Faculty of Medicine, Institute of Biology, Montpellier, France.

"The Mode of Action and the Use of Oral Antidiabetic Compounds" – K. Lundbaek, Mediziniske Universitatetsklinik, Kommune Hospital, Aarhus, Denmark.

"The Pharmacology of Chlorpropamide" – Jurg A. Schneider, E. D. Salgado, and D. A. Jaeger, Pfizer Therapeutic Institute, Maywood, N. J.

"Hypoglycemic Sulfonylureas: Effect of Structure on Activity" – William M. McLamore, G. M. Fanelli, S. Y. P'an, and G. D. Laubach, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

"The Physical Properties of Chlorpropamide and Its Determination in Human Serum" – Thomas Toolan and Richard Wagner, Jr., Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

"The Metabolic Fate of Chlorpropamide in Man" – Philip Johnson, Allen Hennes, and Kelly West, University of Oklahoma, Oklahoma City, Okla.

"The Relative Potencies of Chlorpropamide and Tolbutamide in Man" – Kelly M. West and Stanley R. McCampbell, University of Oklahoma, Oklahoma City, Okla.

"The Effects of the Administration of Chlorpropamide upon the Carbohydrate Metabolism of Isolated Tissues" – D. W. Clarke, University of Toronto, Toronto, Ont., Canada.

"The *in Vivo* Effect of Chlorpropamide on Certain Liver Enzymes in Rats" – Martin D. Sass and Milton B. Handelsman, Veterans Administration Hospital, Brooklyn, N. Y., and State University of New York, N. Y.

"Effects of Chlorpropamide *in Vitro* Compared with Those of Other Hypoglycemic Agents" – Albert Renold, Gaston Zahnd, Bernard Jeanrenaud, and Buris Boshell, Harvard Medical School, Peter Bent Brigham Hospital, and Baker Clinic Research Laboratories, New England Deaconess Hospital, Boston, Mass.

METABOLIC STUDIES

Session Chairman: Max Miller
Western Reserve University, Cleveland, Ohio

2:00 P.M. –

"The Effect of Tolbutamide on the Diabetogenic Action of Somatotropin" – I. A. Mirsky, University of Pittsburgh, Pittsburgh, Pa.

"Insulin Degradation and Factors Influencing It" – Robert Williams, University of Washington, Seattle, Wash.

"Determination of the Rate of Insulin Destruction *in Vivo*" – Thaddeus E. Prout and Ieuan E. Evans, Johns Hopkins University, Baltimore, Md.

"A Comparison of the Influence of Tolbutamide and Small Doses of Insulin on the Splanchnic Output and Peripheral Uptake of Glucose in Man" – James W. Craig, William R. Drucker, Max Miller, and Hiram Woodward, Jr., Western Reserve University, Cleveland, Ohio.

"The Relationship Between the Mechanism of Action of the Sulfonylureas and the Secretion of Insulin into the Portal Circulation" – Leonard Madison, Roger Unger, and Norman Kaplan, The University of Texas Southwestern Medical School, and the Veterans Administration Hospital, Dallas, Tex.

"Insulin- and Tolbutamide-Induced Changes in the Glucose Output of the Liver" – Per Schambye and Finn Tarding, Novo Terapeutisk Laboratorium A/S, Copenhagen, Denmark.

"A Comparison of the Metabolic Effects of Insulin, Chlorpropamide, and Other Hypoglycemic Substances" – Piero Fòa, Giorgio Galansino, Giu-

seppe D'Amico, and Doris Kanameishi, The Chicago Medical School, Chicago, Ill.

"Effect of Chlorpropamide on Intermediary Metabolism in Human Subjects" – Harold Dobson, Hooshang Guilak, and James Greene, Baylor University College of Medicine, Houston, Tex.

"Metabolic Effects of Sulfonylurea Derivatives in Selected Diabetic Subjects" – Joseph L. Izzo, University of Rochester Medical Center, Rochester, N. Y.

"Metabolic Studies of Chlorpropamide in Normal Men and in Diabetic Subjects" – Raymond E. Knauff, Stefan S. Fajans, Eduardo Ramirez, and Jerome W. Conn, University of Michigan Medical School, Ann Arbor, Mich.

FRIDAY, SEPTEMBER 26, 1958

CLINICOMETABOLIC STUDIES

Session Chairman: Edward Tolstoi
Cornell University Medical College, New York, N. Y.

9:00 A.M. –

"Report of Studies with Chlorpropamide in Patients with Diabetes" – Alexander Marble, William Hadley, and Avedis Khachadurian, Joslin Clinic and Baker Clinic Research Laboratory, New England Deaconess Hospital, Boston, Mass.

"The Use of Chlorpropamide in Diabetes Mellitus in the Usual Diabetic Patients and in the Primary and Secondary Tolbutamide Failures" – Samuel Sugar, Lawrence Thomas, and Serkis Tatlier, George Washington University Division, District of Columbia General Hospital, Washington, D. C., and Prince George's General Hospital, Cheverly, Md.

"A Laboratory and Clinical Study of Chlorpropamide in Ambulatory Diabetics" – Milton B. Handelsman, Leon Levitt, and Matthew F. Calabretta, Long Island College Hospital, Brooklyn, N. Y.

"Experiences with Tolbutamide and Chlorpropamide in Tuberculous Diabetic Patients" – Francis Segarra, David Sherman, and Benson Charif, Boston Tuberculosis Sanatorium, Mattapan, Mass.

"The Role of a Tolbutamide Tolerance Test in the Detection of the Mild Diabetic State: A Preliminary Report" – Harold Zarowitz and Benjamin Eis, Jewish Hospital of Brooklyn, Brooklyn, N. Y.

"A Clinical Comparison of Chlorpropamide and Tolbutamide" – George J. Magid, David E. Dorosin, Herbert C. Moffitt, and Peter H. Forsham, University of California Medical School, San Francisco, Calif. and National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.

"Experiences with Chlorpropamide with Reference to its Intermittent Use" – Farid Fuleihan and Calvin H. Plimpton, American University of Beirut, Beirut, Lebanon.

"Clinical Experience with Chlorpropamide" – Barnett Greenhouse, Grace-New Haven Community Hospital, New Haven, Conn.

CLINICOMETABOLIC STUDIES

Session Chairman: Martin Goldner
Jewish Chronic Disease Hospital, Brooklyn, N. Y.

2:00 P.M. –

"Clinical and Pharmacological Comparison of Chlorpropamide and Other Sulfonylureas" – J. M. Stowers, R. B. Hunter, and Lorna W. Constable, University of St. Andrews, Dundee, Scotland.

"One Year of Clinical Experience with Chlorpropamide in More than One Hundred Diabetic Patients" – Zeb Burrell, Jr., Alberto Martinez, and Lenette Burrell, Milledgeville State Hospital, Milledgeville, Ga.

"The Correlation Between Oral Dosage, Blood Levels, and Clinical and Metabolic Activity of Chlorpropamide in the Treatment of Diabetes Mellitus" – Samuel B. Beaser, Harvard Medical School, Boston, Mass.

"Clinical Experiences with Chlorpropamide, Especially in Relation to Previous Carbutamide and Tolbutamide Therapy" – H. A. Heinsen, Waldkrankenhaus, Zeven/Aspe, Germany.

"Clinical Observations with Chlorpropamide in Diabetes Mellitus" – Garfield G. Duncan and Guy L. Schless, University of Pennsylvania, Pennsylvania Hospital, Benjamin Franklin Clinic, Philadelphia, Pa.

"Observations on the Mode of Action and Clinical Effects of Chlorpropamide in Diabetes Mellitus" – Carroll M. Leevy, Abraham Tornow, Philip Greenberg, and Myra Zinke, Seton Hall University, Jersey City, N. J.

"Clinical Experiences with Chlorpropamide: A Double Blind Study" – Charles T. Lee, Jr., Guy L. Schless, and Garfield G. Duncan, University of Pennsylvania and Pennsylvania Hospital, Philadelphia, Pa.

"Long-Term Therapy with Chlorpropamide" – A. W. Alvi and J. P. MacLean, St. Boniface Hospital, St. Boniface, Manitoba, Canada.

PAPERS TO BE READ BY TITLE ONLY

"Chlorpropamide and Glucose Metabolism of Human Skin" – Bernard Appel, Mauray J. Tye, and Gladys Friedler, Tufts University and The Boston City Hospital, Boston, Mass.

"Clinical Evaluation of Chlorpropamide in Diabetes Mellitus" – Ismael Canessa, Sergio Valiente, and Iris Mella, Hospital San Juan de Dios, Santiago, Chile.

"The Role of the Liver in the Early Treatment of Diabetes with P-607" – C. Cardenas-Pupo, University of Havana, Havana, Cuba.

"Effect of Chlorpropamide (P-607) in Diabetic Patients and Normal Subjects" – L. J. Cardonnet, J. J. Staffieri, D. Eberhardt, and R. R. Berli, Institute of Endocrinology and Nutrition, British Hospital, Rosario, Argentina.

"Blood Levels of Chlorpropamide in Normal Men Following Chronic Administration" – Michael Carlozzi, Domenic Iezzoni, and Lawrence Silver, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

"Sulfodrogas en el Tratamiento de la Diabetes" – P. Landabure, Carlos Delbue, Rafael Camerini-Davalos, Carlos Alvarina, Nestor Serrantes, Gregorio Waissman (Buenos Aires), Adolfo Loredo (Cordoba), Enrique Dussel and Fernando Chiquitti (Mendoza), and A. Cabarrou (La Plata), Instituto Nacional de Nutricion, Argentina.

"Preliminary Clinical and Experimental Studies with Chlorpropamide in Diabetes Mellitus" – Ewen Downie, Joseph Bornstein, and Harald Breidahl, University of Melbourne, Australia.

"The Effect of Chlorpropamide on Alloxan Diabetes in Mice" – Francisco Grande and Carlos Martinez, University of Minnesota, Minneapolis, Minn.

"The Effects of Tolbutamide and Chlorpropamide on Patients Exhibiting Jaundice as a Result of Previous Chlorpropamide Therapy" – Harvey Hamff, Harold Ferris, Edwin Evans, and Harold Whiteman, Emory University, Emory University, Ga.

"Studies on the Use of Chlorpropamide in Patients with Diabetes Mellitus" – N. Sheldon Skinner, Jr. and S. Richardson Hill, Jr., University of Alabama Medical Center and the Veterans Administration Hospital, Birmingham, Ala.

"Clinical Experience with Chlorpropamide in the Management of Diabetes Mellitus" – A. Gorman Hills and William A. Abelove, University of Miami School of Medicine, Miami, Fla.

"Clinical and Experimental Studies of Chlorpropamide in Diabetes Mellitus, in Normal Individuals and in Nondiabetics with Hepatic Disease" – B. Knick, University Medical Clinic, Mainz, Germany.

"Experiences with Chlorpropamide, Especially in the Brittle Diabetic" – Joseph Lowenthal, Carl Ross, and William Tully, Duval Medical Center, Jacksonville, Fla.

"A Preliminary Metabolic and Clinical Evaluation of Chlorpropamide" – J. M. Baertl, J. E. Owens, and G. J. Gabuzda, Western Reserve University School of Medicine at Cuyahoga County Hospital, Cleveland, Ohio.

"Cloropropamida – Una Nueva Sulfonilurea en el Tratamiento de la Diabetes. Estudio de 29 Casos" – Alfredo Piazza and Walter Chanamé, Hospital Obrero Lime, Lima, Peru.

"Preliminary Report on the Treatment of Diabetis Mellitus with P-607" – M. Ruiz-Guia, Venezuelan Diabetic Association Center, Caracas, Venezuela.

"Considerations on Control Norms to Evaluate Results of Treatment in Diabetis Mellitus" – M. Ruiz-Guia, Venezuelan Diabetic Association Center, Caracas, Venezuela.

"The Clinical Results of the Treatment of Diabetes Mellitus with Chlorpropamide" – Ignácio de Salcedo and Filomeno Borges, University of Oporto, Portugal.

"Tolerance for Alcohol in Patients on Chlorpropamide" – Saverio Signorelli, Istituto Patalogia, University of Catania, Catania, Italy.

"The Treatment of Diabetes Mellitus in Areas of Nutritional Deficiency" – Agerico B. M. Sison, Angel A. Florentin, and Herminio J. Germar, University of the Philippines, Manila, Philippines.

"The Problem of Diabetes in the Chinese Population and the Impact of Oral Antidiabetics on its Management" – Ho Yuen, Singapore General Hospital, Singapore, Malaya.

"Clinicotherapeutic Evaluation of Chlorpropamide, New Sulfonylurea Derivate, in the Treatment of Diabetes Mellitus" – P. Serrano, National Institute of Cardiology and Hospital Colonia, Mexico, D.F., Mexico.

SATURDAY, SEPTEMBER 27, 1958

CLINICOMETABOLIC STUDIES

Session Chairman: Garfield Duncan
University of Pennsylvania, Pennsylvania Hospital, and Benjamin Franklin Clinic, Philadelphia, Pa.

9:00 A.M. –

"Clinical Studies with Chlorpropamide" – Edward Tolstoi, Cornell University Medical College, New York, N. Y.

"The Use of Chlorpropamide in Brittle and Poorly Controlled Diabetes Mellitus" – Harold Dobson, Hooshang Guilak, R. E. Carter, Hunter Montgomery, and James Greene, Baylor University College of Medicine, Houston, Tex.

"Advantages and Disadvantages in Changing Diabetic Patients from Tolbutamide to Chlorpropamide" – Josef Blöch and Arnold Lenhardt,

Department for Metabolic Diseases, Krankenhaus der Stadt Wien-Lainz, Vienna, Austria.

"Chlorpropamide in the Management of the Adult Diabetic Patient" – Norman W. Drey, Barrett L. Taussig, Jim Waterfield, and Robert Rubin, The Research Institute of The Jewish Hospital of St. Louis, St. Louis, Mo.

"Sites and Intensity of Chlorpropamide Action" – Thaddeus S. Danowski, University of Pittsburgh, Pittsburgh, Pa.

"The Influence of Chlorpropamide upon Spontaneous and Postpancreatectomy Diabetes" – Kermit Pines, Medical College, Columbia University, New York, N. Y.

"The Effects of Chlorpropamide on Endocrine Function in Patients with Diabetes Mellitus and Its Effects in Other Endocrine Disorders" – George J. Hamwi, Thomas G. Skillman, Fred Kruger, and Lucy R. Freedy, Department of Medicine, Ohio State University, Columbus, Ohio.

"Clinical Experience in the Use of N-Propyl-N'-(*p*-chlorobenzenesulfonyl)-urea (P-607) in the Treatment of Diabetes Mellitus" – J. A. García Reyes, J. A. Peña, E. Barroso, and F. Anton, Hospital de Nutricion, Mexico, D. F., Mexico.

"A Summation of the Symposium" – Charles H. Best, Charles H. Best Institute, Toronto, Ont., Canada.

SECOND CONFERENCE

on

PHYSICOCHEMICAL MECHANISM OF NERVE ACTIVITY

Thursday, October 2, and
Friday, October 3, 1958

and

SECOND CONFERENCE

on

MUSCULAR CONTRACTION

Friday, October 3, and
Saturday, October 4, 1958

SECOND CONFERENCE ON PHYSICOCHEMICAL MECHANISM OF NERVE ACTIVITY

Conference Chairman: David A. Nachmansohn
College of Physicians and Surgeons, Columbia University, New York, N. Y.

THURSDAY, OCTOBER 2, 1958

PHYSICAL AND CHEMICAL ASPECTS OF NERVE IMPULSE CONDUCTION

Session Chairman: David A. Nachmansohn
College of Physicians and Surgeons, Columbia University, New York, N. Y.

9:00 A.M. –
Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences; New York University, New York, N. Y.

Introductory Remarks – David A. Nachmansohn, College of Physicians and Surgeons, Columbia University, New York, N. Y.

"Ion Movements During Nerve Activity" – Andrew F. Huxley, Cambridge University, Cambridge, England.

"Synaptic Excitation and Inhibition" – John C. Eccles, The John Curtin School of Medical Research, Australian National University, Canberra, Australia.

"Is the Resting Potential of Ranvier Nodes a Potassium Potential?" – Robert Staempfli, Physiologisches Institut der Universität des Saarlandes, Saarbrücken, The Saar, Germany.

Session Chairman: Ernst Chain
Istituto Superiore di Sanità, Rome, Italy

2:00 P.M. –

"Ion Movement Studied with Single Isolated Electroplax" – Ernest Schoffeniels, College of Physicians and Surgeons, Columbia University, New York, N. Y.

"Design of a Nerve Gas Antidote on the Basis of Molecular Complementariness" – Irwin B. Wilson, College of Physicians and Surgeons, Columbia University, New York, N. Y.

"Recent Progress in Intermediary Carbohydrate Metabolism of Brain and Muscle" – Ernst Chain, Istituto Superiore di Sanità, Rome, Italy.

FRIDAY, OCTOBER 3, 1958

NEUROMUSCULAR AND SYNAPTIC TRANSMISSION

Session Chairman: Yngve Zotterman
Kungl. Veterinärhögskolan, Stockholm, Sweden

9:00 A.M. –

"Structure of the Neuromuscular Junction" – René Couteaux, Laboratoire de Biologie Animale, University of Paris, Paris, France.

"Some Ionic Factors which Influence the Action of Acetylcholine at the Muscle End Plate Membrane" – William L. Nastuk, College of Physicians and Surgeons, Columbia University, New York, N. Y.

"The Presynaptic Element in Neuromuscular Transmission" – Walter F. Riker, Cornell University Medical College, New York, N. Y.

"Studies on the Mechanism of Curarisation" – Carlos Chagas, Instituto de Biofisice, University of Brazil, Avenue Pasteur, Rio de Janeiro, Brazil.

SENSORY RECEPTORS

Session Chairman: Robert Staempfli
Physiologisches Institüt der Universität des Saarlandes, Saarbrücken, The Saar, Germany

2:00 P.M. –

"Nervous Mechanism of Taste" – Yngve Zotterman, Kungl. Veterinärhögskolan, Stockholm, Sweden.

"The Generation of Electric Activity in a Nerve Ending" – Werner R. Loewenstein, College of Physicians and Surgeons, Columbia University, New York, N. Y.

SECOND CONFERENCE ON MUSCULAR CONTRACTION

Honorary Chairman: Wallace O. Fenn

Conference Chairman: Alexander Sandow
New York University, New York, N. Y.

FRIDAY, OCTOBER 3, 1958

MUSCLE FIBERS AND FIBRILS

Session Chairman: Wallace O. Fenn
University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

8:00 P.M. –
Introductory Remarks – Alexander Sandow, New York University, New York, N. Y.

"Structural Basis of Contraction Mechanism in Muscle" – H. E. Huxley and Jean Hanson, University College, London, England.

"The Relaxation of the Contracted Actomyosin System" – Hans H. Weber, Max Planck Institute for Physiology, Heidelberg, Germany.

SATURDAY, OCTOBER 4, 1958

EXCITATION-CONTRACTION COUPLING

Session Chairman: Alexander Sandow
New York University, New York, N. Y.

9:00 A.M. –
"Impulse Propagation and Refractory Period in Striated Muscle Fibers" – Fritz Buchtal, Institute of Neurophysiology, University of Copenhagen, Copenhagen, Denmark.

"Local Activation of Muscle" – Andrew F. Huxley, Cambridge University, Cambridge, England.

"Studies on Excitation-Contraction Coupling" – Arpad Csapo, The Rockefeller Institute for Medical Research, New York, N. Y.

MECHANOCHEMISTRY

Session Chairman: Teru Hayashi
Columbia University, New York, N. Y.

2:00 P.M. –

"The Energy Supply for Muscle Contraction" – R. E. Davies, D. Cain, and A. M. Delluva, University of Pennsylvania Medical School, Philadelphia, Pa.

"Muscular Contraction and Response of Cytochrome Chain" – Britton Chance, Eldridge Reeves Johnson Foundation for Medical Research, University of Pennsylvania, Philadelphia, Pa.

"Relaxing Factor of Muscle" – John Gergely, Massachusetts General Hospital, Boston, Mass.

Discussant: Franz F. Jobsis, Eldridge Reeves Johnson Foundation for Medical Research, University of Pennsylvania, Philadelphia, Pa.

TROPHOBLAST AND ITS TUMORS

Friday, October 10 and
Saturday, October 11, 1958

Conference Chairman: William B. Ober
Knickerbocker Hospital, College of Physicians and Surgeons, Columbia University, Albert Einstein College of Medicine, Yeshiva University, New York, N. Y.

FRIDAY, OCTOBER 10, 1958

FUNDAMENTAL CONCEPTS

Session Chairman: Carl G. Hartman
Ortho Research Foundation, Raritan, N. J.

9:00 A.M. –

Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences, New York University, New York, N. Y.

Introduction to the Conference:

"Historical Perspectives on Trophoblast and Its Tumors" – William B. Ober, Knickerbocker Hospital, College of Physicians and Surgeons, Columbia University, Albert Einstein College of Medicine, Yeshiva University, New York, N. Y.

"The Biology of Trophoblast" – Bent G. Böving, Carnegie Institution of Washington, Department of Embryology, Baltimore, Md.

"Proliferative Changes in the Trophoblast Following Ovariectomy and Fetectomy in the Rat" – Robert W. Noyes, Stanford University School of Medicine, New Haven, Conn.

"Hormonal Actions of Chorionic Gonadotrophin" – Joseph T. Velardo, Yale University School of Medicine, New Haven, Conn.

Discussion Leader: Carl G. Hartman, Ortho Research Foundation, Raritan, N. J.

HYDATIDIFORM MOLE

Session Chairman: Hugh G. Grady
Seton Hall College of Medicine and Dentistry, Jersey City, N. J.

2:00 P.M. –

"Genesis of Hydatidiform Mole: Old and New Concepts" – Henry W. Edmonds, Washington Hospital Center, Washington, D. C.

"Problems in the Classification of Hydatidiform Mole" – J. Smalbraak, Haarlem, The Netherlands.

"Management of Patients with Hydatidiform Mole" – C. Lee Buxton, Yale University School of Medicine, New Haven, Conn.

"Chorionic Gonadotrophin Determinations in Patients with Hydatidiform Mole and Choriocarcinoma"–Eleanor Delfs, The Johns Hopkins Hospital, Baltimore, Md.

Discussion Leader: Hugh G. Grady, Seton Hall College of Medicine and Dentistry, Jersey City, N. J.

SATURDAY, OCTOBER 11, 1958

TROPHOBLASTIC TUMORS

Session Chairman: John I. Brewer
Northwestern University School of Medicine, Chicago, Ill.

9:00 A.M. –

"Chorioadenoma Destruens" – Ronald R. Greene, Northwestern University School of Medicine, Chicago, Ill.

"Choriocarcinoma in the Female" – W. Wallace Park, Queen's College, (University of St. Andrews), Dundee, Scotland.

"Choriocarcinoma in the Male" – Nathan B. Friedman, Cedars of Lebanon Hospital and University of Southern California, Los Angeles, Calif.

Discussion Leader: John I. Brewer, Northwestern University School of Medicine, Chicago, Ill.

Session Chairman: Gordon W. Douglas
New York University College of Medicine, New York, N. Y.

2:00 P.M. –

"Geographic Variation in the Occurrence of Hydatidiform Mole, and Choriocarcinoma" – Lalla Iverson, Armed Forces Institute of Pathology, Washington, D. C.

"Natural History of Choriocarcinoma Problems of Immunity and Spontaneous Regression" – Wadi A. Bardawil and Benjamin L. Toy, St. Margaret's Hospital, Boston, Mass.

"Chemotherapy of Choriocarcinoma and Related Trophoblastic Tumors" – Roy Hertz, Delbert M. Bergenstal, Mortimer B. Lipsett, and Ralph Reisfeld, National Cancer Institute, Public Health Service, Bethesda, Md.

Discussion Leader: Gordon W. Douglas, New York University College of Medicine, New York, N. Y.

ENZYMES OF POLYNUCLEOTIDE METABOLISM

Thursday, October 16,
Friday, October 17, and
Saturday, October 18, 1958

Conference Chairman: Jay S. Roth
Hahnemann Medical College, Philadelphia, Pa.

THURSDAY, OCTOBER 16, 1958

CRYSTALLINE PANCREATIC RIBONUCLEASE

Session Chairman: C. B. Anfinsen, Jr.
National Heart Institute, Public Health Service, Bethesda, Md.

9:00 A.M. –

Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences, New York, N. Y.; New York University, New York, N. Y.

Introductory Remarks – C. B. Anfinsen, Jr. National Heart Institute, Public Health Service, Bethesda, Md.

"The Relationship of Structure to Function in Ribonuclease" – Fred H. White, Jr. and Christian B. Anfinsen, National Heart Institute, Public Health Service, Bethesda, Maryland.

"Immunochemical Reactions Involving Ribonuclease" – Ray K. Brown, J. Durieux, R. Delaney, E. Leikhim, and B. J. Clark, Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.

"Some Properties of Yeast Ribonucleic Acid and Ribonuclease" – George Kalnitsky, J. P. Hummel, C. Dierks, H. Resnick, L. B. Barnett, and J. R. Carter, College of Medicine, State University of Iowa, Iowa City, Iowa.

Discussants: Sherman R. Dickman, University of Utah College of Medicine, Salt Lake City, Utah.
Anwar Hakim, Miami Heart Institute, Miami, Fla.
William F. Harrington, National Heart Institute, Public Health Service, Bethesda, Md.
C. W. H. Hirs, Rockefeller Institute for Medical Research, New York, N. Y.
Fred Karush, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

INTRACELLULAR RIBONUCLEASES

Session Chairman: Jay S. Roth
Hahnemann Medical College, Philadelphia, Pa.

2:00 P.M. –

Introductory Remarks – Jay S. Roth, Hahnemann Medical College, Philadelphia, Pa.

"Studies in the Distribution of Intracellular Ribonucleases" – Gaston de Lamirande and Claude Allard, Montreal Cancer Institute Research Laboratories, Montreal, Canada.

"Chromatographic Investigations of Mouse Pancreas Ribonucleases" – Sherman R. Dickman and Gene A. Morrill, University of Utah College of Medicine, Salt Lake City, Utah.

"Purification and Characterization of the Ribonucleases of Calf Spleen" – Mary E. Maver, Elbert A. Peterson, Herbert A. Sober, and Antoinette E. Greco, Laboratory of Biochemistry, National Institutes of Health, Public Health Service, Bethesda, Md.

"Comparative Studies on Tissue Ribonucleases" – Jay S. Roth, Hahnemann Medical College, Philadelphia, Pa.

"Liver Ribonucleases" – Eric Reid and James T. Nodes, Chester Beatty Research Institute, Royal Marsden Hospital, London, England.

Discussants: C. B. Anfinsen, Jr., National Heart Institute, Public Health Service, Bethesda, Md.
L. A. Heppel, National Institute or Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.
Anwar Hakim, National Childrens' Cardiac Hospital, Miami, Fla.

Moderator: C. B. Anfinsen, Jr.
National Heart Institute, Public Health Service, Bethesda, Md.

8:00 P.M. –

Round Table Discussion – "The Relationship of Enzyme Heterogeneity to Specificity"

FRIDAY, OCTOBER 17, 1958

POLYNUCLEOTIDE PHOSPHORYLASES

Session Chairman: L. A. Heppel
National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

9:00 A.M. –

Introductory Remarks – L. A. Heppel, National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.; and Severo Ochoa, New York University College of Medicine, New York, N. Y.

"Mechanism of Action of Polynucleotide Phosphorylase" – L. A. Heppel, National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

"An Examination of the Mechanism of Action of Polynucleotide Phosphorylase Through a Study of the Activating and Inhibitory Effects of Polynucleotide-Binding Dyes, Mg, and Ionic Strength" – Roland F. Beers, Jr., Robert W. Johnson Laboratory, The Children's Hospital, Baltimore, Md.

"The Effect of End Groups and the Initial Site of Attack on Polynucleotides by Polynucleotide Phosphorylase and Certain Phosphodiesterases" – Russell Hilmoe, National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

Discussants: Severo Ochoa, New York University College of Medicine, New York, N. Y.
E. S. Canellakis, Yale University School of Medicine, New Haven, Conn.
Edward Herbert, Massachusetts Institute of Technology, Cambridge, Mass.

THE STRUCTURE AND PROPERTIES OF SYNTHETIC AND OTHER POLYNUCLEOTIDES

Session Chairman: Severo Ochoa
New York University College of Medicine, New York, N. Y.

2:00 P.M. –

Introductory Remarks – Severo Ochoa, New York University College of Medicine, New York, N. Y.

"The Properties of Synthetic Polyribonucleotides and RNA in Solution" – Paul A. Doty, Harvard University, Cambridge, Mass.

"The Molecular Structure and Chemical Reactivity of the Synthetic Polyribonucleotides" – Alexander Rich, Massachusetts Institute of Technology, Cambridge, Mass.

"Electron Microscopy of Polynucleotides" – Cecil E. Hall, Massachusetts Institute of Technology, Cambridge, Mass.

Discussants: Jacques Fresco, Harvard University, Cambridge, Mass.
Severo Ochoa, New York University College of Medicine, New York, N. Y.
James Watson, Harvard University, Cambridge, Mass.
Robert F. Steiner, Naval Medical Research Institute, Bethesda, Md.

Moderator: Sherman R. Dickman
University of Utah College of Medicine, Salt Lake City, Utah

8:00 P.M. –
Round Table Discussion – "Assay of Ribonuclease Activity"

SATURDAY, OCTOBER 18, 1958

ENZYMES OF POLYDESOXYRIBONUCLEOTIDE METABOLISM

Session Chairman: M. Laskowski
Marquette University School of Medicine, Milwaukee, Wis.

9:00 A.M. –
"The Enzymic Synthesis of Desoxyribonucleic Acid" – I. R. Lehman, Washington University School of Medicine, St. Louis, Mo.

"Studies on the Chemical Synthesis and Enzymatic Degradation of Deoxyribo-oligonucleotides" – G. M. Tener, P. T. Gilham, W. E. Razzell, and H. G. Khorana, British Columbia Research Council, Vancouver, B.C., Canada.

"Enzymes Hydrolyzing DNA" – M. Laskowski, Marquette University School of Medicine, Milwaukee, Wis.

Discussants: S. Zamenhoff, Columbia University College of Physicians and Surgeons, New York, N. Y.
L. Cunningham, Marquette University School of Medicine, Milwaukee, Wis.
F. J. Bollum, McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.
G. Schmidt, Tufts University School of Medicine, Boston, Mass.
A. Dounce, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Hans G. Boman, University of Uppsala, Uppsala, Sweden.
L. A. Heppel, National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

ORGANIZING COMMITTEE

Jay S. Roth, *Chairman*

C. B. Anfinsen, Jr.
L. A. Heppel
M. Laskowski
Severo Ochoa

RADIOPAQUE DIAGNOSTIC AGENTS

Friday, October 24 and
Saturday, October 25, 1958

Conference Chairman: Maxwell H. Poppel
New York University-Bellevue Medical Center, New York, N. Y.

FRIDAY, OCTOBER 24, 1958

Session Chairman: Harold G. Jacobson
Montefiore Hospital, New York, N. Y.

9:00 A.M. –

Greetings from the Academy – Albert S. Gordon, Chairman, Section of Biology, The New York Academy of Sciences, New York, N. Y.; New York University, New York, N. Y.

"Contrast Media Research – General Aspects" – Vernon H. Wallingford, Mallinckrodt Chemical Works, St. Louis, Mo.

"Chemical Aspects of Radiopaque Compounds" – Sidney Archer, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

"Some Pharmacological Aspects of Radiopaque Compounds" – James O. Hoppe, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

"The Pathologist and Toxicologist in the Safety Evaluation and Methods Employed in the Development of Radiodiagnostic Compounds" – Frederick Coulston, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

"Chelates and Rare Earths" – Robert Shapiro and Domenick Papa, The Hospital of St. Raphael, New Haven, Conn.

"Metal Chelates as Possible Roetgenographic Contrast Media" – Martin Rubin, Georgetown University, Washington, D. C. and Giovanni Di Chiro, National Institutes of Health, Washington, D. C.

"A New Category of Contrast Media: Radiopaque, Water-Soluble, Polyvalent Metallic Chelates" – Robert M. Nalbandian, William Rice, and Warren O. Nickel, Wayne State University, Detroit, Mich.

"Uniform Colloidal Dispersions for Hepatolienography" – Robert J. Dummel and Sydney F. Thomas, Palo Alto Medical Clinic, Palo Alto, Calif.

Session Chairman: John A. Evans
New York Hospital – Cornell Medical Center, New York, N. Y.

2:00 P.M. –

"Lymphangiography and Lymphadenography with Various Contrast Agents" – Harry W. Fischer, State University of Iowa, Iowa City, Ia.

"Iopanoic Acid" – Walter M. Whitehouse, University Hospital, Ann Arbor, Mich.

"Two-Phase Opacification of the Liver in Cirrhosis" – Francis F. Ruzicka, Jr., St. Vincent's Hospital, New York, N. Y.

"Pancreatography" – Henry Doubilet, Maxwell H. Poppel, and John H. Mulholland, New York University-Bellevue Medical Center, New York, N. Y.

"Experience with the Avoidance of Allergic Reactions From a Pyelographic Medium by the Use of Antihistamine Drugs" – Reed M. Nesbit, University of Michigan, Ann Arbor, Mich.

"Duografin for Simultaneous Intravenous Studies of the Urinary and Biliary Tracts" – William H. Shehadi, New York Polyclinic Medical School, New York, N. Y.

"Rose Bengal and Iodopyracet as Radiodiagnostic Agents in Liver and Kidney Diseases" – George V. Taplin, O. M. Meredith, Jr., Chester C. Winter, and Delores Johnson, University of California at Los Angeles, Veterans Administration Center, Los Angeles and Harbor General Hospital, Torrance, Calif.

SATURDAY, OCTOBER 25, 1958

Session Chairman: John F. Roach
Albany Medical School and Hospital, Albany, N. Y.

9:00 A.M. –

"The Evaluation of Physiological Responses to Arterial Administrations of Various Contrast Media" – Robert A. Moe and Bradford N. Craver, Squibb Institute for Medical Research, New Brunswick, N. J.

"Application of Cinefluorography to Modern X-Ray Diagnosis" – Joseph Jorgens, Veterans Administration Hospital, Minneapolis, Minn.

"Stereo-Serio Angiography" – J. M. Sanchez-Perez, Mexico, D.F., Mexico.

"Percutaneous Retrograde Femoral Arteriography" – Billy P. Sammons, John R. Williams, and Charles Gartenlaub, U. S. Naval Hospital, St. Albans, L. I., N. Y.

"Intravenous Aortography" – Richard H. Greenspan, Eugene Bernstein, and Merle K. Loken, University of Minnesota, Minneapolis, Minn.

"High Concentrations of Diatrizoate Methylglucamine" – J. Stauffer Lehman, Philadelphia, Pa.

Session Chairman: William H. Shehadi
New York Polyclinic Medical School, New York, N. Y.

2:00 P.M. –

"Large Volume Myelography" – Eugene Tainter and Charles E. Grayson, Sutter Community Hospitals, Sacramento, Calif.

"Oral Diatrizoate Methylglucamine" – Harold G. Jacobson and Jerome H. Shapiro, Montefiore Hospital, New York, N. Y.

"Diatrizoate Sodium" – Frederick A. Lloyd, Northwestern University, Chicago, Ill.

"Diprotrizoate Sodium" – Majic S. Potsaid and L. L. Robbins, Massachusetts General Hospital, Boston, Mass.

"Thioxokon, a New Urethrographic Agent" – David W. Goddard, Daytona Beach, Fla.

"Nephrotomography" – John A. Evans, New York Hospital-Cornell Medical Center, New York, N. Y.

"Hysterosalpingography Employing a New Water-Soluble Medium: Diatrizoate Iodipamide Methylglucamine" – William J. Sweeney, III, New York Hospital, New York, N. Y.

NEW MEMBERS

Elected June 19, 1958

LIFE MEMBERSHIP

Itoh, Heihachi, M.D., Viral Diseases. Research Associate, Kitasato Institute for Infectious Diseases, Tokyo, Japan.

Richardson, Howard Lockhart, M.D., Cancer Research. Pathologist, Institute of Biological Research, Seattle, Wash.

SUSTAINING MEMBERSHIP

Burket, Walter Cleveland, M.D., Biological, Medical Sciences. Attending Staff, The Alexian Brothers Hospital, Evanston, Ill.

Cole, Versa V., M.D., Pharmacology. Staff Physician, Caro State Hospital for Epileptics, Caro, Mich.

Gemoets, Henry Napoleon, M.D., Internal Medicine. Practicing Physician, Houston, Texas.

Gordon, Arthur, M.D., Internal Medicine, Cardiology. Visiting Physician, Queens General Hospital, Flushing, N. Y.

Gray, Ralph E., M.D., General Surgery. Chairman, Surgical Committee, Dow Hospital, Freeport, Texas.

Holman, Winston Churchill, M.D., Radiology. Radiologist, St. Mary's Hospital, Port Arthur, Texas.

Katz, Cecelia, M.D., Psychoanalysis. Private Practice, Chicago, Ill.

Markson, Victor I., M.D., Internal Medicine. Private Practice, Beaver Falls, Pa.

Painter, John Milton, M.D., Internal Medicine, Allergy. Assistant Clinical Professor, Western Reserve University, Cleveland, Ohio.

Pawel, Ernst Siegfried, M.D., Internal Medicine, Physiology. Practicing Internist, Akron, Ohio.

Rosenberg, Benjamin, M.D., Cardiovascular Disease. Associate Attending, Internal Medicine, Maimonides Hospital, Brooklyn, N. Y.

Rosenhain, Bruno, M.D., Internal Medicine. Physician, Forest Hills, N. Y.

Rush, Paul W., M.D., Industrial Medicine. Medical Director, Corn Products Refining Company, Argo, Ill.

Saperstein, Morris, M.D., Psychosomatic Medicine. Private Practice, Muncie, Ind.

Schimel, John L., M.D., Social Psychiatry. Director, Psychiatry, Hebrew Home for the Aged, Riverdale, N. Y.

Schwartz, Emanuel K., Ph.D., Psychology. Assistant Dean, Postgraduate Center for Psychotherapy, New York, N. Y.

Singerman, Leonard J., M.D., General Medicine. Generalist, Pleasure Ridge Park, Ky.

ACTIVE MEMBERSHIP

Adams, Adolph A., M.D., Radiology. Practicing Radiologist, New York, N. Y.

Adams, D. M., M.D., Medicine. Physician, Adams Hospital, Inc., Panama City, Fla.

Adelson, Sidney L., M.D., Endocrinology. Chief, Medical Out-Patient, Harper Hospital, Detroit, Mich.

Allen, Herbert C. Jr., M.D., Nuclear Medicine. Director, Radioisotope Department, The Methodist Hospital, Houston, Texas.

Allen, John D., M.D., Medicine. Pathologist, Director, Laboratories, Kentucky Baptist Hospital, Louisville, Ky.

Anagnostakos, Nicholas P., M.S., Physiology. Biochemist, Lederle Laboratories, American Cyanamid Company, Pearl River, N. Y.

Andrews, David B., Ph.D., Chemistry, Chemical Engineering. Manager, Process Engineering, Dyestuff & Chemical Division, Westfield, N. J.

Andrews, George Reid, M.D., Psychiatry. Clinical Director, Raleigh State Hospital, Raleigh, N. C.

Atwater, John Spencer, M.D., Medicine. Physician, Atlanta, Ga.

Avola, Francis A., M.D., General Surgery. Private Practice, Medford, Mass.

Back, Kenneth Charles, Ph.D., Pharmacology. Senior Scientist, Warner-Lambert Research Institute, Morris Plains, N. J.

Bagnall, James, M.D., Physiology. Physiology Division, Brookhaven National Laboratory, Upton, N. Y.

Bailey, Percy William, Jr., M.D., Neurology, Psychiatry. Private Practice, Beaumont, Texas.

Baker, Jeannette L., M.D., Geriatrics. Geriatric Psychiatry, State Hospital, Ferqus Falls, Minn.

Bates, David Hinrichs, M.D., Internal Medicine. Private Practice, Woodstock, Conn.

Beasley, Ernest W. Jr., M.D., Internal Medicine, Endocrinology. Associate, Emory University School of Medicine, Atlanta, Ga.

Black, Jack, M.D., Clinical Research. Physician, Clinical Research Division, Schering Corporation, Bloomfield, N. J.

Blom, Gaston Eugene, M.D., Psychoanalysis, Child Psychiatry. Associate Professor, Psychiatry, University of Colorado, Denver, Colo.

Boardman, Donnell W., M.D., Internal Medicine. Senior Associate, Acton Medical Associates, Acton, Mass.

Bolster, R. H., M.D., General Medicine, Allergy. Department of Allergy, Bryn Mawr Hospital, Bryn Mawr, Pa.

Borow, Edward, M.D., Internal Medicine, Private Practice, Bound Brook, N. J.

Borrus, Joseph C., M.D., Neuropharmacology. Consulting Psychiatrist, Rutgers University, New Brunswick, N. J.

Botwinick, Isaiah S., A.B., Chemistry. Director, Research Testing Laboratories, New York, N. Y.

Bradner, William T., Ph.D., Microbiology. Assistant Staff Member, Sloan-Kettering Institute, New York, N. Y.

Breazeale, Edward L., M.S., Toxicology. Director, Arizona Serological Laboratories, Tucson, Ariz.

Brownlee, Inez E., M.S., Bacteriology, Parasitology. Instructor, University of Oregon Medical School, Portland, Oreg.

Busby, Carroll Lee, M.D., Biology. Director, Medical Services, Mississippi State Hospital, Whitfield, Miss.

Bustin, Andrew G., M.D., Inorganic Metabolism. Practicing Physician, Joliet, Ill.

Cadogan, W. P., Sc.D., Chemistry. Manager, Research & Development, American Machine & Foundry Company, Stamford, Conn.

Cahan, Jacob M., M.D., Medicine. Instructor, Jefferson Medical College, Philadelphia, Pa.

Capozzella, Henry F., M.D., Anesthesiology. Director, Anesthesiology, Arlington Hospital, Arlington, Va.

Carthage, Patricia Linden, B.S., Microbiology. Assistant Scientist, Warner-Lambert Research Institute, Morris Plains, N. J.

Case, Robert, B.Sc., Pharmacology. Chief Cancer Research Pharmacist, Roswell Park Memorial Institute, Buffalo, N. Y.

Chess, Stella, M.D., Child Psychiatry. Associate Clinical Professor, New York Medical College, New York, N. Y.

Christian, Robert T., Ph.D., Virology. Research Associate, Obstetrics-Gynecology Department, University of Michigan Medical School, Ann Arbor, Mich.

Clinton, Marshall, M.D., Endocrinology. Assistant Attending Physician, Buffalo General Hospital, Buffalo, N. Y.

Cohen, Burton D., M.D., Internal Medicine. Cardiology Resident, Second (Cornell) Medical Division, Bellevue Hospital, New York, N. Y.

Cohen, Irvin M., M.D., Psychiatry. Associate Professor, University of Texas Medical Branch, Houston, Texas.

Cook, Freeman W., Ph.D., Morphologic Hematology, Poultry Science. Assistant Professor, North Carolina State College, Raleigh, N. C.

Corpe, William A., Ph.D., Microbiology, Biochemistry. Assistant Professor, Barnard College, New York, N. Y.

Corrado, Albert G., M.D., Allergy. Physician, Medical Arts Building, Richland, Wash.

Cotton, Claire Eugene, M.D., Internal Medicine. Associate Physician, St. Vincent Charity Hospital, Cleveland, Ohio.

Creager, Ray O., M.D., Child Psychiatry. Director, Kalamazoo Child Guidance Clinic, Kalamazoo, Mich.

Creveling, Robert L., M.D., Anatomical, Clinical Pathology. Resident, Pathology, Letterman Army Hospital, Presidio of San Francisco, Calif.

Darlington, Horace F., M.D., Medicine. Medical Staff Member, Memorial & Chester Company Hospital, West Chester, Pa.

Dasler, Adolph F., M.D., Neurology, Psychiatry. Private Practice, Psychiatry, Muskegon, Mich.

Davis, J. Galvin, M.D., Otorhinolaryngology. Professor, University of Nebraska College of Medicine, Omaha, Nebr.

Decker, Clarence F., Ph.D., Biochemistry. Assistant Scientist, Argonne National Laboratory, Lemont, Ill.

Defoney, George F., M.Sc., Pharmacy. Analytical Chemist, Merck Sharp & Dohme Company, Philadelphia, Pa.

De Robertis, Eduardo D. P., M.D., Histology, Cytology. Professor, Institute of Histology & Embryology, Buenos Aires, Argentina.

Direnga, Barbara Ann, B.S., Microbiology. Assistant Scientist, Warner-Lambert Research Institute, Morris Plains, N. J.

Dmytryk, Eugene T., M.D., Surgery. Senior Instructor, St. Louis University, St. Louis, Mo.

Dolan, Gerald J., M.D., Medicine. General Practioner, Hillsdale, N. J.

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